



02

Molecular biology

## Essential ideas

- 2.1 Living organisms control their composition by a complex web of chemical reactions.
- 2.2 Water is the medium of life.
- 2.3 Compounds of carbon, hydrogen, and oxygen are used to supply and store energy.
- 2.4 Proteins have a very wide range of functions in living organisms.
- 2.5 Enzymes control the metabolism of the cell.
- 2.6 The structure of DNA allows efficient storage of genetic information.
- 2.7 Genetic information in DNA can be accurately copied and can be translated to make the proteins needed by the cell.
- 2.8 Cell respiration supplies energy for the functions of life.
- 2.9 Photosynthesis uses the energy in sunlight to produce the chemical energy needed for life.

Computer image of an insulin molecule. Insulin is a peptide (protein) hormone that helps to regulate glucose levels between the bloodstream and the cytoplasm of cells.

Organic chemistry is the chemistry of carbon compounds. Biochemistry is the branch of organic chemistry that attempts to explain the chemistry characteristics of living organisms. Even though biochemistry can be amazingly complex and varied, there are common patterns that are well known. For example, all living organisms are made up of molecules that can be classified into one of four types:

- carbohydrates
- lipids
- proteins
- nucleic acids.

In addition, biochemical processes in living organisms follow certain common pathways for which we can study the common pattern. So, when we study cell respiration or photosynthesis as biochemical processes, we do not need to study a completely different process for each organism or species.

This chapter will introduce you to some of the more common biochemically important molecules and processes.

## 2.1 Molecules to metabolism

### Understandings:

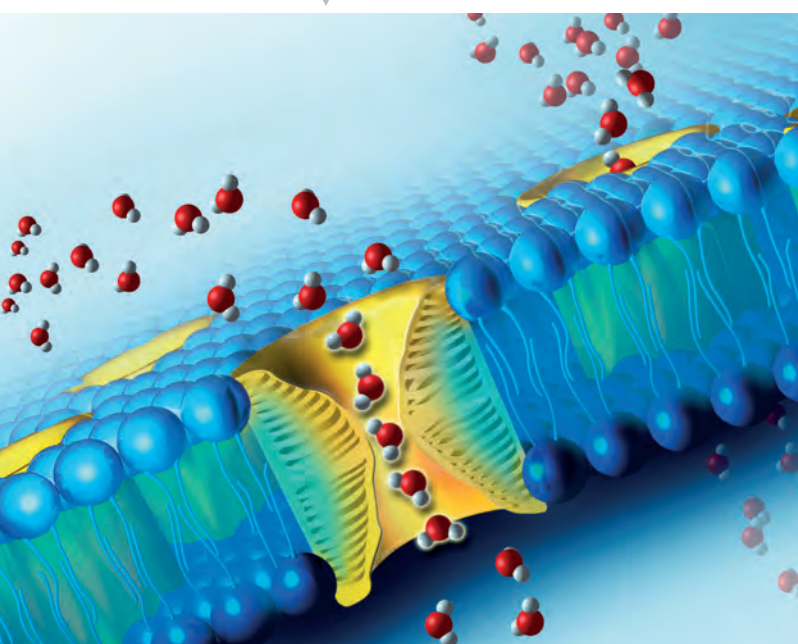
- Molecular biology explains living processes in terms of the chemical substances involved.
- Carbon atoms can form four covalent bonds, allowing a diversity of stable compounds to exist.
- Life is based on carbon compounds, including carbohydrates, lipids, proteins, and nucleic acids.
- Metabolism is the web of all the enzyme-catalysed reactions in a cell or organism.
- Anabolism is the synthesis of complex molecules from simpler molecules, including the formation of macromolecules from monomers by condensation reactions.
- Catabolism is the breakdown of complex molecules into simpler molecules including the hydrolysis of macromolecules into monomers.



### NATURE OF SCIENCE

Falsification of theories: the artificial synthesis of urea helped to falsify vitalism.

Aquaporins are channels that allow water molecules to pass through the membrane.



### Applications and skills:

- Application: Urea as an example of a compound that is produced by living organisms but can also be artificially synthesized.
- Skill: Drawing molecular diagrams of glucose, ribose, a saturated fatty acid, and a generalized amino acid.
- Skill: Identification of biochemicals such as sugars, lipids, or amino acids from molecular diagrams.

#### Guidance

- Only the ring forms of D-ribose, alpha-D-glucose, and beta-D-glucose are expected in drawings.
- Sugars include monosaccharides and disaccharides.
- Only one saturated fat is expected, and its specific name is not necessary.
- The variable radical of amino acids can be shown as R. The structure of individual R-groups does not need to be memorized.
- Students should be able to recognize from molecular diagrams that triglycerides, phospholipids, and steroids are lipids. Drawings of steroids are not expected.
- Proteins or parts of polypeptides should be recognized from molecular diagrams showing amino acids linked by peptide bonds.

### Molecular biology is the chemistry of living organisms

The majority of molecules within all living organisms can be categorized into one of four biochemical groupings. Those groupings are carbohydrates, lipids, proteins, and nucleic acids. In turn, these four groupings of molecules interact with each other in a wide variety of ways in order to carry out the metabolism of each cell.

Consider the following example of metabolism in order to see how living processes are actually chemical substances interacting in predictable patterns. Insulin is a protein hormone that facilitates the movement of glucose from the bloodstream to the interior of cells. Insulin does this by interacting with protein channels in body cell plasma

membranes, thereby opening those channels to glucose. As long as glucose is in a higher concentration outside the cell compared with inside the cell, glucose will continue to move through the open channel by diffusion. The plasma membrane is largely composed of a type of lipid called a phospholipid. Because of molecular polarity differences, phospholipids will not allow glucose to pass through the membrane without going through the protein channels. Both insulin and the channels within the plasma membrane are proteins, therefore they must both be coded for by deoxyribonucleic acid (DNA) within the cells of the organism in which they are working.

Glucose is a carbohydrate, the phospholipid molecules are lipids, both insulin and the membrane channels are proteins, and DNA is a nucleic acid. Each molecule has a specific function and collectively they all work together in order to ensure that body cells have access to glucose for their energy needs. All the biochemistry within all living organisms can be 'broken down' into smaller interactions similar to the above example.



## CHALLENGE YOURSELF

- 1 Read through this example of molecular interactions leading to a physiological response. After doing so, try to classify each of the *named* molecular components as a carbohydrate, lipid, protein, or nucleic acid.

When a predator, such as a snake, catches and eats a small rodent, one of the main sources of nutrition that the snake is consuming is the muscle of the prey animal. That muscle is primarily composed of two molecules: actin and myosin. When the ingested muscle reaches the intestines of the snake, enzymes (such as trypsin) help the snake digest the actin and myosin into amino acids. Other enzymes (such as lipase) help the snake digest the triglyceride fats within the adipose tissue of the rodent.

## Carbon-based life



Organic chemistry is the study of compounds that contain carbon. Some compounds that contain carbon are not classified as organic, including carbon dioxide. Despite this important exception, there are very many molecules containing carbon that are classified as organic. The molecules already mentioned above (carbohydrates, proteins, lipids, and nucleic acids) are all organic molecules. These are the molecules from which all living things are composed, thus the element carbon can be considered to be the keystone element for life on Earth. This is the reason why you sometimes hear life on Earth being described as ‘carbon based’.

You may recall from your introductory chemistry course that each carbon atom has an atomic number of six. Directly this means that carbon has six protons, but indirectly it also means that carbon has six electrons. Two of these six electrons form the stable inner shell, and four are found in the second and unfilled shell. Carbon’s way of ‘filling’ this second shell of electrons is to share four electrons with other atoms in order to create a stable configuration of eight electrons in total. Each time carbon shares one of its electrons, a covalent bond is formed, and carbon always forms four covalent bonds.

There are many other elements found within the molecules of living organisms. In addition to carbon, the following elements are common: hydrogen, oxygen, nitrogen, and phosphorus. These elements are used in the molecular structures of carbohydrates, proteins, lipids, and nucleic acids by forming covalent bonds with carbon, and very often by forming covalent bonds with each other.

## Biochemical compounds that are important to living organisms

Living things are composed of an amazing array of molecules. We can start to make sense of all of these molecules by classifying them into different types. Molecules of the same type have certain qualities in common and become fairly easy to recognize with a little practice. Table 2.1 shows some of the more common biochemically important molecules and their subcomponents (or building blocks).

Carbon dioxide is one of the very few carbon-containing substances that is not classified as organic. In this model the black, centre atom is the carbon atom, and the two red atoms are the oxygen atoms.

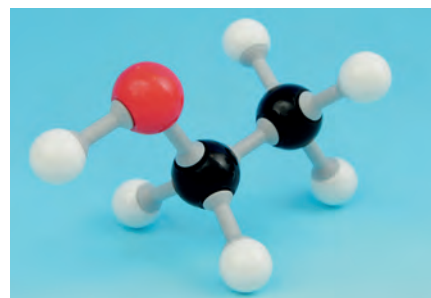


Carbon’s name is derived from the Latin word ‘carbo’, meaning charcoal.

### TOK

You will notice that virtually all the images you see of atoms and molecules are in the form of models. Why are models used? What do the real atoms and molecules look like?

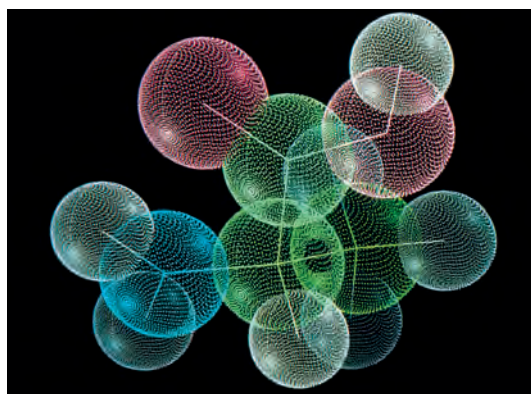
The structure and bonding of an ethanol molecule. The two atoms shown in black are carbon, the red atom is oxygen, and all the white atoms are hydrogen.



**Table 2.1 Types of molecules**

Molecule	Subcomponents (building blocks)
Carbohydrates	Monosaccharides
Lipids	Glycerol, fatty acids, phosphate groups
Proteins (polypeptides)	Amino acids
Nucleic acids	Nucleotides

This is a colour-coded molecular model of the amino acid alanine. Green = carbon; pink = oxygen; blue = nitrogen; white = hydrogen



As you study biochemistry, you will soon learn to recognize and classify common biochemical molecules into appropriate categories. Table 2.2 shows some of the common categories and examples of molecules.

**Table 2.2 Common categories of molecules**

Category	Subcategory	Example molecules
Carbohydrates	Monosaccharides	Glucose, galactose, fructose, ribose
	Disaccharides	Maltose, lactose, sucrose
	Polysaccharides	Starch, glycogen, cellulose, chitin
Proteins		Enzymes, antibodies, peptide hormones
Lipids	Triglycerides	Fat stored in adipose cells
	Phospholipids	Lipids forming a bilayer in cell membranes
	Steroids	Some hormones
Nucleic acids		Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP)

The ways in which these molecules interact with each other in living organisms is amazingly diverse and interesting. All of these interactions are referred to as metabolism and that is the focus of the next section.

## Metabolism: reactions controlled by enzymes

If you were to visualize zooming into the inside of a cell down to the molecular level, you would see thousands of molecules colliding with each other as they move through their aqueous (water-based) environment. Many of these collisions do not result in

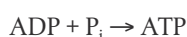
any action other than the molecules changing direction and thus heading into new collisions. But sometimes these molecular collisions provide enough energy to specific molecules, the reactants, for those reactants to undergo a chemical reaction of some type. That single chemical reaction would be one of the millions of reactions that occur within that cell that comprise that cell's metabolism. In a multicellular organism, all of the reactions within all of the cells (and fluids such as blood) comprise the metabolism of the organism.

When two molecules collide, there are a large number of factors that determine whether a reaction occurs or not. Some of these factors include the:

- identity of the colliding molecules
- orientation of the colliding molecules (where they hit each other)
- the speed of the molecules when they collide.

Cells use enzymes in order to increase the likelihood that a collision will lead to a useful reaction. Enzymes are protein molecules that have a specific shape into which a reactant(s) can fit, at a molecular location called the active site of the enzyme. By having an active site, the enzyme increases the likelihood of a reaction.

Let's look at an example of one reaction that makes up part of a typical cell's metabolism. The reaction we will consider is one in which adenosine triphosphate (ATP) is formed or synthesized. ATP is the most common molecule used by cells when chemical energy is required. ATP is synthesized from the bonding of adenosine diphosphate (ADP) to a phosphate (P) group. This reaction requires energy, and that energy may come originally from food (cell respiration) or sunlight (photosynthesis). Put simply, the reaction can be summarized as:



adenosine diphosphate plus inorganic phosphate yields adenosine triphosphate

The odds of these two reactants (ADP + P<sub>i</sub>) colliding at a very high speed, at exactly the correct orientation, leading to a new covalent bond forming between them, is extremely small. That is where an enzyme comes into play: the enzyme acts as a catalyst for the reaction. The catalyst will not be used up and so the enzyme will be available to act as a catalyst many times over. The ADP reactant fits into part of the enzyme's active site, and the inorganic phosphate group reactant fits perfectly oriented next to it, and, within a small fraction of a second, the two reactants become covalently bonded to each other. So, in effect, three molecules are involved in the collision but only two of them result in the production of ATP. The ATP is then released from the active site and the enzyme is ready for another collision with another ADP and phosphate group. The catalysis provided by the enzyme enables this reaction to occur at a much higher reaction rate and with less collisional energy compared with the same reaction occurring without the enzyme.

All of your metabolism is based on this fundamental scenario. A multitude of reactions are occurring inside each living organism's cells at any given moment. Most of these reactions are being catalysed by enzymes. These are the reactions that make up your overall metabolism, and include diverse sets of reactions, including:

- replication of DNA, in preparation for cell division
- synthesis of RNA, allowing chemical communication between the nucleus and cytoplasm



**Metabolism is best thought about from a molecular perspective.** Often, people think only of physiological parameters, such as heart rate and digestion, as their metabolism. But remember that metabolism is all of the reactions within all of the cells of an organism.



The 'collisional energy' referred to in this section is called activation energy. An enzyme is often defined as an organic catalyst that lowers the activation energy of a reaction.

- synthesis of proteins, including bonding of one amino acid to another
- cell respiration, with nutrients being converted into ATP
- photosynthesis, with light energy being used to create carbohydrates
- and many, many more.

Many of the carbon atoms found in the food that you eat (such as carbohydrates) will be eliminated from your body in the molecules of carbon dioxide that you breathe out.

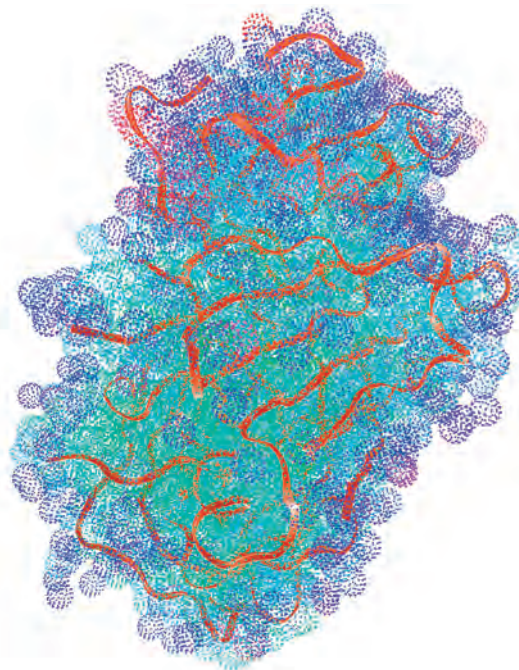


## Metabolism = catabolism + anabolism

It is very common for people to use some form of the word metabolism in everyday conversations, for example: 'I wish I had a higher metabolic rate so that I could eat more without putting on weight'. When people say something like that, they are usually thinking of factors like their heart rate. There is actually a great deal more than this involved in metabolism. As described in the previous section, your metabolism is the sum total of all the enzyme-catalysed reactions taking place within you. Some of these enzyme-catalysed reactions function to convert large, complex molecules (like many of the foods that we eat) to smaller, simpler molecular forms. This is called catabolism. Other enzyme-catalysed reactions carry out the reverse: they convert small, simple molecules into a larger, more complex molecules. This is called anabolism. These molecular conversions are done for a variety of reasons, and we will look at a couple of examples in this section. You will find more examples later as you study the various biochemical and physiological processes common to living organisms.

Many organisms, including all animals, rely on the foods that they eat to obtain the building block molecules that make up their larger molecules. When animals eat foods, the food is digested (or hydrolysed) into the building blocks (catabolism). After these building blocks are transported to body cells, they are bonded together to form larger molecules once again (anabolism).

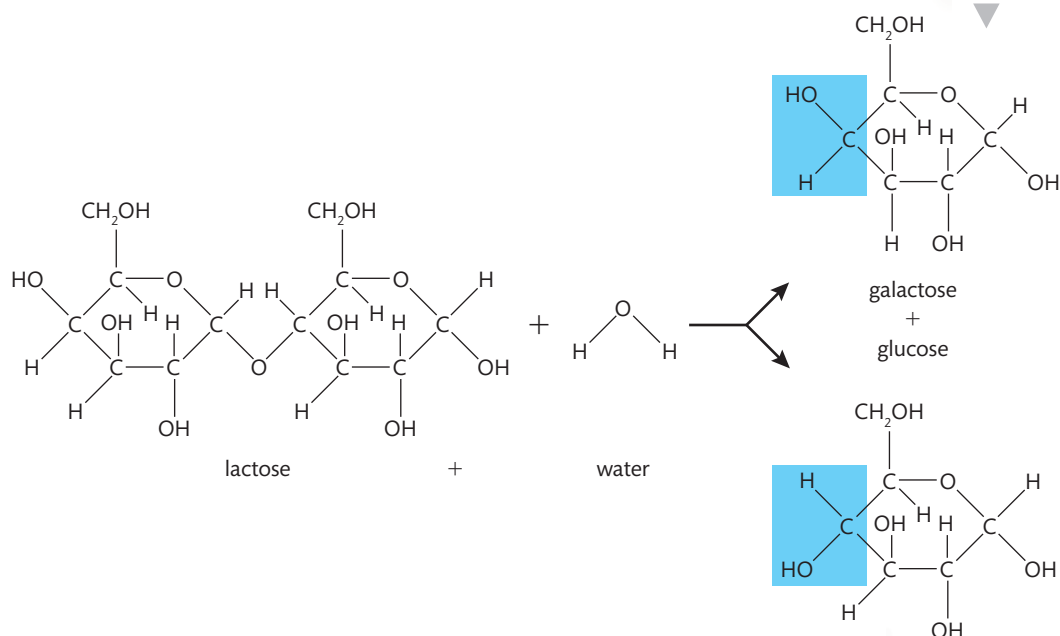
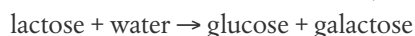
This computer graphic image shows pepsin, an enzyme that helps to digest proteins. Pepsin is an example of a hydrolysing enzyme.



Let's explore what happens to ingested foods. Foods are chemically digested in your alimentary canal. The digestive enzymes that accomplish this are hydrolysing

enzymes. Each reaction is called a hydrolysis and requires a molecule of water as a reactant. This is a good way to recognize hydrolysis reactions: water is always 'split' as part of the reaction. Below are four examples of hydrolysis reactions.

**1** Hydrolysis of a disaccharide to two monosaccharides (see Figure 2.1).

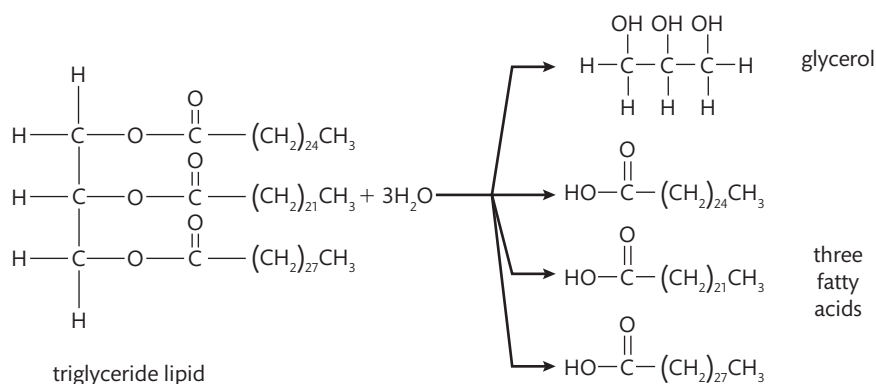
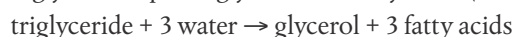


**Figure 2.1** Hydrolysis of the disaccharide lactose to form the two monosaccharides galactose and glucose. The difference between galactose and glucose is shown in the blue areas.

**2** Hydrolysis of a polysaccharide to many monosaccharides.

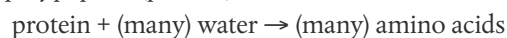


**3** Hydrolysis of a triglyceride lipid to glycerol and fatty acids (see Figure 2.2).



**Figure 2.2** Hydrolysis of a triglyceride lipid to form glycerol and three fatty acid molecules.

**4** Hydrolysis of a polypeptide (protein) to amino acids.





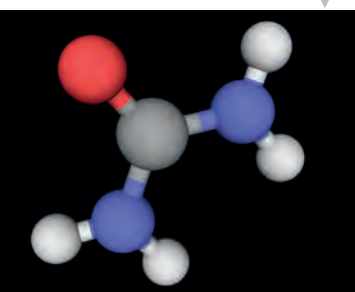
Condensation reactions are, in many ways, the reverse of hydrolysis reactions. In cells, condensation reactions occur to re-form the larger, biochemically important, molecules. In the four examples given above, simply reverse the reaction arrow and each example shows a condensation reaction. For example:

- condensation of amino acids to form a polypeptide  
(many) amino acids  $\rightarrow$  protein + (many) water

Notice that in condensation reactions, water molecule(s) are products rather than reactants. Condensation reactions require a different type of enzyme, one that is capable of catalysing reactions in which covalent bonds are created rather than broken.

In summary, remember that an organism's metabolism comprises all of the reactions that occur within all of its cells. Thus metabolism can also be thought of as the sum of all the reactions that work to hydrolyse large biochemical substances into smaller subcomponents (catabolism), plus all those reactions that rebuild large, more complex biochemical substances from the smaller subcomponents (anabolism).

Molecular model of urea. The large grey atom is carbon. Each of the two blue side-chains is an amine functional group and the red atom is a double-bonded oxygen atom.



The German physician and chemist Friedrich Wöhler.



### NATURE OF SCIENCE

It is difficult for people growing up and learning in today's world to truly appreciate the scientific ideas of the past. One of the philosophies that was widely held nearly two centuries ago was called vitalism. Vitalism was the belief that living organisms and inanimate things differed fundamentally because living organisms contained a non-physical or vitalistic element, and were subject to different principles of nature compared with non-living things. A part of this philosophy even suggested that the organic molecules that are characteristic of living organisms could only be produced within living organisms.

One example of an organic molecule is urea. Urea is produced in some living organisms as a nitrogenous waste product. In mammals, including humans, urea is produced in the liver, enters the bloodstream, and is then filtered out of the bloodstream by the kidneys, and becomes a component of urine. The fundamentals of this process were known in the early 1800s, and it was assumed, because of the widely held principle of vitalism, that this was the only way urea could be produced.

In 1828, Friedrich Wöhler, a German physician and chemist, made a discovery that helped change the thinking behind vitalism. In his laboratory, Wöhler had mixed two inorganic substances, cyanic acid and ammonium, in a beaker. He noticed the formation of a crystalline substance that looked familiar to him. After testing, he confirmed that the crystals were urea. He had previously only come across urea crystals in the study of the compounds that are characteristic of urine. For perhaps the first time in a controlled setting, an organic molecule was synthesized from inorganic substances.

Wöhler did not fully appreciate the meaning and consequences of his findings at the time, but, as it turned out, his published work was soon used as evidence that vitalism should be questioned as a scientific theory. It was not long before other substances, such as amino acids, were synthesized from inorganic precursors in various laboratories.

What does this show about the nature of science?

- Scientific theories undergo modifications over time. Some are just modified, while some are proved to be completely false.
- Frequently, important discoveries are made 'accidentally'. Dr Wöhler did not add the two inorganic substances together with the intention of making urea.
- Frequently, a scientific discovery is not appreciated immediately for its importance. This is one of the reasons why discoveries need to be published. This allows the entire scientific community to fit new knowledge into the bigger picture of science, and sometimes that only happens much later.

## CHALLENGE YOURSELF

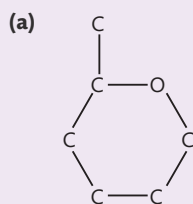
2 Drawing molecular diagrams of common biochemical substances is easier than you might think, especially with a little practice. You will be expected to be able to draw the following molecules from memory:

- alpha-D-glucose
- beta-D-glucose
- ribose
- an unnamed saturated fatty acid
- a generalized amino acid.

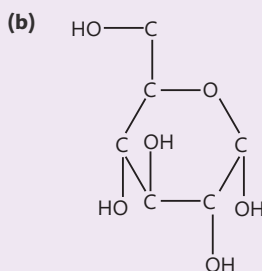
When drawing these, and other complex organic molecules, it helps to draw them in a sequential pattern. That sequence is given below.

- Draw the carbons first (this is called the carbon backbone of the molecule).
- Then add in any functional groups that are found as part of the molecule.
- 'Fill in' with hydrogen atoms, to ensure that all the carbon atoms are showing four covalent bonds.
- Look over your entire structure, to make sure that all of the different atom types are showing the correct number of covalent bonds for that type of element.
- If you know or have been given the chemical formula of the substance, count the number of each type of atom and check that number against the known formula.

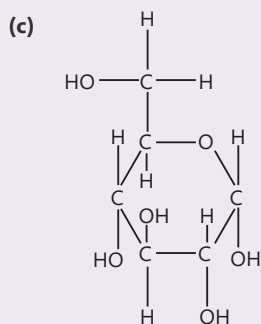
Here is how it would work for the monosaccharide sugar called alpha-D-glucose, a substance that we know has the chemical formula of  $C_6H_{12}O_6$ .



**Figure 2.3** The carbon backbone of alpha-D-glucose.



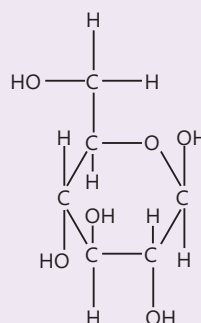
**Figure 2.4** The alcohol groups added.



**Figure 2.5** The hydrogens added.

Note: Be sure to count the covalent bonds around each element, and make sure that the number is appropriate for each. You should also count the number of each type of atom and check that against the known formula of  $C_6H_{12}O_6$ .

Beta-D-glucose has exactly the same chemical formula as alpha-D-glucose and the two are, in fact, isomers of each other. Alpha-D-glucose and beta-D-glucose differ only in how a few of the atoms within the structure are oriented in space in relation to each other. Here is the finished molecular diagram of beta-D-glucose:

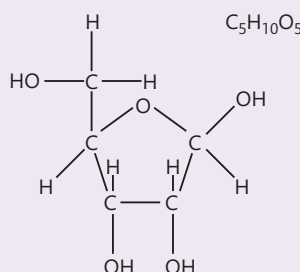


**Figure 2.6** Beta-D-glucose.

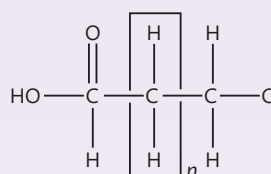
Trying to draw complex organic molecules by somehow memorizing the entire intact structure is frustrating and impossible for most people. Instead, always use the sequence of steps shown on the previous page, of laying out the carbon backbone, adding the functional groups, and then filling in with hydrogen(s) as needed. This will not only help you learn the molecules you need to know, but it will also enable you to look at large, complex biochemical molecules from a new and more useful perspective.



Here are the completed molecular diagrams of another three molecules that you need to learn to draw from memory. Get out some paper and a pencil and practise drawing the two glucose molecules shown on the previous page and the three molecules shown below. Don't practise drawing them in their entirety, but use the step-by-step process as shown above. Do this until you are confident that you know each one very well.

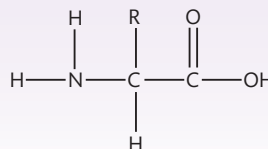


◀ **Figure 2.7** D-ribose.



◀ **Figure 2.8** A generalized fatty acid.

Where  $n$  = any number between 3 and 29 (11–23 are the most common)



◀ **Figure 2.9** A generalized amino acid.

Where R = 1 of 20 variable groups

## Exercises

- 1 One way to check whether organic molecules are drawn correctly is to make a sketch based on the information given and then count the number of atoms of each element using a given or known formula. Draw each of the molecules described below and then check each against the formula given in the answers.
  - (a) Sketch a single carbon atom, add an alcohol group, fill in with hydrogen atoms. Give the formula of the molecule.
  - (b) Sketch a single carbon atom, add an amine group, add a carboxyl group, fill in with hydrogens. Give the formula of the molecule.
- 2 Give the products of each of the following reactions:
  - (a) the complete hydrolysis of a starch molecule
  - (b) the condensation reaction between glucose and galactose
  - (c) the complete hydrolysis of a triglyceride lipid.
- 3 Briefly describe the two aspects of metabolism.

## 2.2 Water

### Understandings:

- Water molecules are polar and hydrogen bonds form between them.
- Hydrogen bonding and dipolarity explain the cohesive, adhesive, thermal, and solvent properties of water.
- Substances can be hydrophilic or hydrophobic.

### Applications and skills:

- Application: Comparison of the thermal properties of water with those of methane.
- Application: Use of water as a coolant in sweat.
- Application: Modes of transport of glucose, amino acids, cholesterol, fats, oxygen, and sodium chloride in blood in relation to their solubility in water.

#### Guidance

- Students should know at least one example of a benefit to living organisms of each property of water.
- Transparency of water and maximum density at 4°C do not need to be included.
- Comparison of the thermal properties of water and methane assists in the understanding of the significance of hydrogen bonding in water.



#### NATURE OF SCIENCE

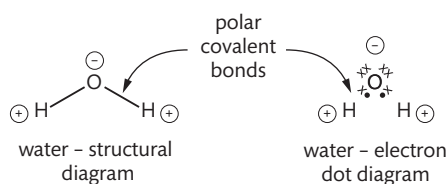
Use theories to explain natural phenomena: the theory that hydrogen bonds form between water molecules explains the properties of water.

## The structure of water molecules and the resulting polarity

Water is the solvent of life. Living cells typically exist in an environment in which there is water within the cell (cytoplasm) and also water in the surrounding environment (intercellular fluid, fresh or salt water, etc.). We refer to all solutions as aqueous solutions if water is the solvent, no matter what mixture of substances make up the solutes. Thus, cytoplasm and water environments such as the oceans are all aqueous solutions.

In order to understand the many properties of water, and the importance of those properties to living organisms, we must first consider the structure of water molecules.

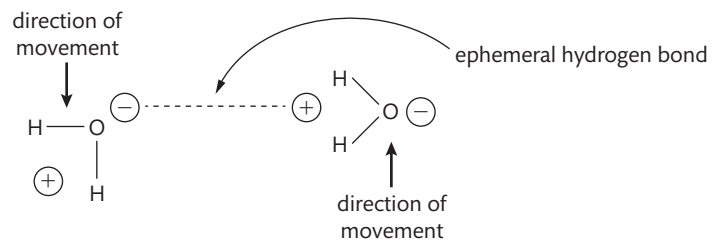
The covalent bonds between the oxygen atom and the two hydrogen atoms of a single water molecule are categorized as polar covalent bonds. You should remember from fundamental chemistry that covalent bonds form when two atoms share electrons. As electrons are negatively charged and the nucleus of an atom (because of the protons) is positively charged, any electrons that are shared equally create a bond and, because the charges cancel, this is called a non-polar covalent bond. The bond between two carbons is a good example of this type of bond. Polar covalent bonding results from an unequal sharing of electrons. In water, the single oxygen atom is bonded to two different hydrogen atoms. Each oxygen–hydrogen bond is a polar covalent bond, and results in a slight negative charge at the oxygen end of the molecule and a slight positive charge at the end with the two hydrogens. Because of the triangular shape of a water molecule, the two ends of each molecule have opposite charges, with



**Figure 2.10** The shared electrons between oxygen and hydrogen are not shared equally, thus they are polar covalent bonds. This gives rise to the polarity of water.



**Figure 2.11** In liquid water, water molecules form 'split second' hydrogen bonds with other water molecules (dotted line), despite the fact that water continues to move in many different directions. These short-lived hydrogen bonds give rise to many of the interesting properties of water.



### Cohesive properties

Water molecules are highly cohesive. Cohesion is when molecules of the same type are attracted to each other. As mentioned earlier, water molecules have a slightly positive end and a slightly negative end. Whenever two water molecules are near each other, the positive end of one attracts the negative end of another; this is hydrogen bonding. When water cools below its freezing point, the molecular motion has slowed to the point where these hydrogen bonds become locked into place and an ice crystal forms. Liquid water has molecules with a much faster molecular motion, and the water molecules are able to influence each other, but not to the point where molecules stop their motion. The ephemeral hydrogen bonding between liquid water molecules explains a variety of events, including:

- why water forms into droplets when it is spilt
- why water has a surface tension that allows some organisms to 'walk on water' (for some this is 'run on water')
- how water is able to move as a water 'column' in the vascular tissues of plants.



### Adhesive properties

Water molecules are certainly not the only molecules in nature that exhibit polarity. Any attraction between two unlike molecules is called adhesion. Thus when water

You can float a paper clip on water because of the surface tension of water. Make sure you maximize the surface area of the paper clip on the water if you try this.



A water strider making use of the high surface tension of water.

molecules are attracted to cellulose molecules by hydrogen bonding, the attraction is an example of adhesion because the hydrogen bonding is between two different kinds of molecules. Where is this important in nature? One example is the column of water in plant vascular tissue, mentioned above. Cohesion and adhesion are both at work, because the water molecules exhibit cohesion to each other, and they also exhibit adhesion to the inside of the vascular tubes, which are partially composed of cellulose. When the column of water is 'pulled up', cohesion moves each molecule up a bit; when the column is not being 'pulled up', adhesion keeps the entire column from dropping down within the tube. The same phenomenon occurs when water is placed in a capillary tube; in fact, you can think of the vascular tissue in plants as being biological capillary tubes.

Water evaporates from leaves through small openings called stomata. As shown here, each stoma has two cells, called guard cells, that surround it. When the guard cells swell with water, the stoma appears between the cells, and water evaporates through the stoma. One benefit to the plant of this is the cooling effect that evaporation provides.



## Thermal properties

Water has thermal properties that are important to living things. One of those thermal properties is high specific heat. In simple terms, this means that water can absorb or give off a great deal of heat without changing temperature very much. Think of a body of water on a very cold night: even though the air may be very cold, the body of water is relatively stable in temperature. All living things are composed of a great deal of water, and so you can think of your water content as a temperature stabilizer. Water also has a high heat of vaporization. This means that water absorbs a great deal of heat when it evaporates. Many organisms, including ourselves, use this as a cooling mechanism. Your internal body heat results in perspiration, and the perspiration then evaporates from your skin. Much of the heat that turned the water molecules from the liquid phase to the vapour phase came from your body, and thus sweating not only makes you feel cooler, it really does lower your temperature.

## Solvent properties

Water is an excellent solvent of other polar molecules. You may remember from earlier science classes that like dissolves like. The vast majority of molecules typically found inside and outside most cells are also polar molecules. This includes carbohydrates, proteins, and nucleic acids (DNA and RNA). Most types of lipids are relatively non-polar and thus most organisms have special strategies to deal with the transport and biochemistry of lipids.

Because water is an excellent solvent for biochemically important molecules, it is also the medium in which most of the biochemistry of a cell occurs. A cell contains a wide variety of fluids, all of which are primarily water. We refer to such solutions as aqueous solutions. Table 2.3 shows some common aqueous solutions in which specific biochemical reactions take place.



The word 'stoma' comes from the Greek word meaning mouth or opening. In medicine, stoma is a surgically created opening in the body that replaces a normal opening.



Basilisk lizards may be as long as 0.8 m, but they can run across the surface of bodies of water. The relatively large surface area of their toes does not break through the surface tension of the water as long as they keep running.



**Specific heat is the amount of heat per unit mass required to raise the temperature one degree Celsius.**



**Heat of vaporization is the amount of heat required to convert a unit mass of liquid into vapour with no increase in temperature.**

**Table 2.3 Common aqueous solutions**

Aqueous solution	Location	Common reactions
Cytoplasm	Fluid inside cells but outside organelles	Glycolysis/protein synthesis reactions
Nucleoplasm	Fluid inside nuclear membranes	DNA replication/transcription
Stroma	Fluid inside chloroplast membranes	Light-independent reactions of photosynthesis
Blood plasma	Fluid in arteries, veins, and capillaries	Loading and unloading of respiratory gases/clotting

### Examples of water as a solvent in plants and animals

The properties of water make it an excellent medium for transport. Vascular tissue in plants carries water and a variety of dissolved substances. More specifically, xylem carries water and dissolved minerals up from the root system to the leaves of a plant. Phloem then transports dissolved sugars from the leaves to the stems, roots, and flowers of a plant.

Blood is the most common transport medium in animals, and is largely made up of water. The liquid portion of blood is called blood plasma. Some of the more common solutes in blood plasma are:

- glucose (blood sugar)
- amino acids
- fibrinogen (a protein involved in blood clotting)
- hydrogen carbonate ions (as a means of transporting carbon dioxide).

### Water ‘loving’ or water ‘fearing’ substances

Molecules in living systems interact with water in a variety of ways. Remember that water is the solvent of life, and living cells typically have an aqueous environment both inside and outside their plasma membrane.

Molecules, such as water, that are polar substances are said to be hydrophilic, or water ‘loving’. The majority of substances that are biochemically important are polar. Polar molecules easily dissolve in water, because a polar solvent will dissolve polar solutes. It is not difficult to recognize most of the molecules that are hydrophilic, as these molecules typically contain functional groups that result in the molecules being polar. Carbohydrates are a good example of polar molecules; their relative solubility in water is attributed to their multiple hydroxyl (alcohol) functional groups.

Molecules that are classified as non-polar are said to be hydrophobic, or water ‘fearing’. Organic substances that are non-polar are typically composed of just carbons and hydrogens (hydrocarbons) or have large areas of the molecule where there are only carbons and hydrogens. Methane ( $\text{CH}_4$ ) is an example of a hydrophobic molecule; it is composed of only one carbon and four hydrogens. Methane will not dissolve in water. Examples of biochemically important molecules that are predominately non-polar are the fatty acids found in triglyceride lipids and phospholipids. In addition to a carboxyl functional group at one end, a fatty acid consists of a long chain of carbons

Try doing a web search on the topic of ‘memory of water’. Are any of the claims you find examples of pseudoscience rather than science?

**TOK**



with only hydrogens. The carboxyl group gives the fatty acid slight polarity at that end, but the chain of hydrocarbons is so long that the majority of the molecule is non-polar and thus hydrophobic.

Protein molecules can be differentially polar depending on the arrangement of their amino acids. Some amino acids are relatively polar and some are non-polar. The location of each type of amino acid is important within the three-dimensional structure of the protein. Good examples are the proteins that attach into and extend out of a cell membrane. The amino acids making up the portion of the protein that attaches to (and extends down into) the membrane are hydrophobic and easily mix with the hydrophobic fatty acid 'tails' of the membrane phospholipid molecules. The portion of the protein that extends out of the membrane is predominately made up of hydrophilic amino acids that easily mix with the water environment either inside or outside the cell or organelle.

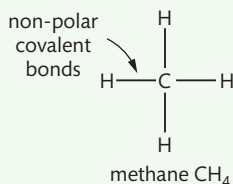
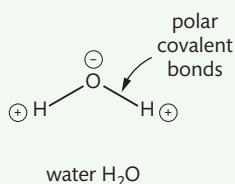


▲ This photo shows what happens when a hydrophobic substance encounters water. The two types of molecules do not mix because they are not soluble.

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You probably already know the freezing point ( $0^{\circ}\text{C}$ ) and boiling point ( $100^{\circ}\text{C}$ ) of water. You may not already know the phase change temperatures for methane: the freezing point of methane is  $-183^{\circ}\text{C}$  and the boiling point is  $-162^{\circ}\text{C}$ .

It is interesting to think about why these two substances have such very different phase change temperatures. Consider the structure and polarity of these two molecules.



The polar covalent bonds within water give rise to the polarity of the molecule. All of the covalent bonds within methane are non-polar and so methane is a non-polar substance. All molecules composed of just carbons and hydrogens (hydrocarbons) are non-polar.

When methane undergoes a phase change, because of its lack of polarity, there are no hydrogen bonds that influence the change of phase. You have probably realized that methane has a very low (cold) freezing point and also a very low boiling point. When methane changes from a liquid to a gas at  $-162^{\circ}\text{C}$  there are no hydrogen bonds attracting the molecules to each other. Thus they 'escape' from each other with only a relatively small amount of molecular motion needed. That is not true for water molecules: each water molecule is constantly forming, breaking, and almost instantly reforming hydrogen bonds with other water molecules. When water changes from a liquid to a gas at  $100^{\circ}\text{C}$ , the high temperature is necessary to create the relatively high rate of molecular motion needed to enable the molecules to 'escape' from each other.

When methane changes from its liquid phase to its solid phase (at its freezing point,  $-183^{\circ}\text{C}$ ), the change in phase is explained by the fact that methane no longer has enough molecular motion to exist as a liquid. Water makes this phase change at a much higher temperature ( $0^{\circ}\text{C}$ ) because, when the molecular rate of motion becomes low enough, hydrogen bonding locks water molecules into stable geometric forms known as ice crystals.

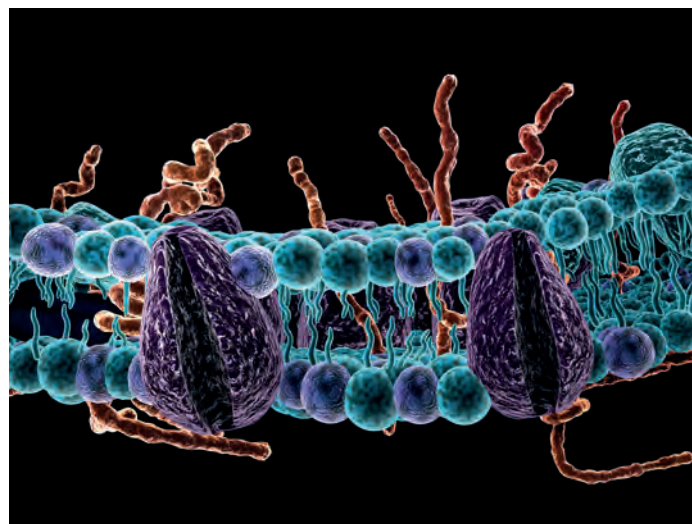
We cannot actually see the hydrogen bonds. However, the theory that is used to explain hydrogen bonding is largely supported by many pieces of evidence, including those described above. Sometimes, in the nature of science, a theory helps explain a phenomenon and then multitudes of similar phenomena support the theory.



**Figure 2.12** A comparison of water and methane.



An artist's drawing of a cell membrane with proteins. The portions of the proteins found within the bilayer of phospholipids are composed of relatively non-polar amino acids, whereas those outside the bilayer are composed of many polar amino acids.



A typical person might be able to survive about 3 weeks without food. However, a typical person would only survive 1 week or less without any water intake.



### How does solubility in water affect the mode of transport of molecules in organisms?

Water in living organisms acts as a mode of transport for the variety of molecules that must be moved about both within cells and between cells. Just think of the various water-based fluids you already know, such as cytoplasm, intercellular fluid, blood, and digestive juices. Because of their different polarities, each type of substance has a different solubility in whatever aqueous environment it is found in, including blood plasma. Table 2.4 summarizes the various relative polarities of a few selected molecules and shows whether or not an alternative mode of transport is needed as that substance circulates in the bloodstream.

**Table 2.4 Polarity of different molecules**

Substance	High or low relative solubility in water	Mode of transport in an aqueous environment (no special mode means the substance dissolves directly and easily into water)
Glucose	Polar molecule/high solubility	No special mode of transport needed/dissolves directly in aqueous plasma
Amino acids	Varying polarity but all are reasonably soluble	No special mode of transport needed/dissolve directly in aqueous plasma
Cholesterol	Largely non-polar/very low solubility	Transported by blood proteins that have polar amino acids on the outer portion to give water solubility, and non-polar amino acids internally to bind the non-polar cholesterol
Fats	Non-polar fatty acid components/very low solubility	Transported by blood proteins that have polar amino acids on the outer portion to give water solubility, and non-polar amino acids internally to bind the non-polar fatty acid molecules
Oxygen	Travels as diatomic O <sub>2</sub> /low solubility	Relatively low solubility in water is exacerbated by the relatively high temperature of warm-blooded animals (oxygen is less soluble in warm aqueous solutions)/haemoglobin is used to bind and transport oxygen molecules reversibly
Sodium chloride	Ionizes/high solubility	No special mode of transport needed/sodium chloride is an ionic compound, it ionizes into separately charged Na <sup>+</sup> and Cl <sup>-</sup> ions in aqueous plasma

## Exercises

- 4 Choose any specific aquatic or terrestrial animal and make a list of all the ways in which water is important to that animal.
- 5 How are the properties of water involved in any item of your list?

## 2.3 Carbohydrates and lipids

### Understandings:

- Monosaccharide monomers are linked together by condensation reactions to form disaccharides and polysaccharide polymers.
- Fatty acids can be saturated, monounsaturated, or polyunsaturated.
- Unsaturated fatty acids can be *cis* or *trans* isomers.
- Triglycerides are formed by condensation from three fatty acids and one glycerol.

### Applications and skills:

- Application: Structure and function of cellulose and starch in plants and glycogen in humans.
- Application: Scientific evidence for health risks of *trans* fats and saturated fatty acids.
- Application: Lipids are more suitable for long-term energy storage in humans than carbohydrates.
- Application: Evaluation of evidence and the methods used to obtain the evidence for health claims made about lipids.
- Skill: Use of molecular visualization software to compare cellulose, starch, and glycogen.
- Skill: Determination of body mass index by calculation or use of a nomogram.

#### Guidance

- The structure of starch should include amylose and amylopectin.
- Named examples of fatty acids are not required.
- Sucrose, lactose, and maltose should be included as examples of disaccharides produced by combining monosaccharides.

## Monosaccharides: the building blocks of disaccharides

Biochemically important molecules can be extremely large and complex but they are always made of smaller monomer (building block) molecules. The monomers of carbohydrates are the monosaccharides. At the beginning of this chapter you were introduced to hydrolysis reactions and an opposite set called condensation reactions. Condensation reactions are key to that part of your metabolism called anabolism, where larger molecules are synthesized from smaller monomer units. As the monomer units of carbohydrates are monosaccharides, we will start by looking at their structure. Monosaccharides can be classified according to how many carbon atoms they contain. The three most common monosaccharides are:

- trioses, containing 3 carbons and with the chemical formula  $C_3H_6O_3$
- pentoses, containing 5 carbons and with the chemical formula  $C_5H_{10}O_5$
- hexoses, containing 6 carbons and with the chemical formula  $C_6H_{12}O_6$ .

You may have noticed a common pattern in the formulas of these three simple sugars: monosaccharides typically fit the formula  $C_nH_{2n}O_n$ , where  $n$  equals the number of carbon atoms.



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Evaluating claims: health claims made about lipids in diets need to be assessed.



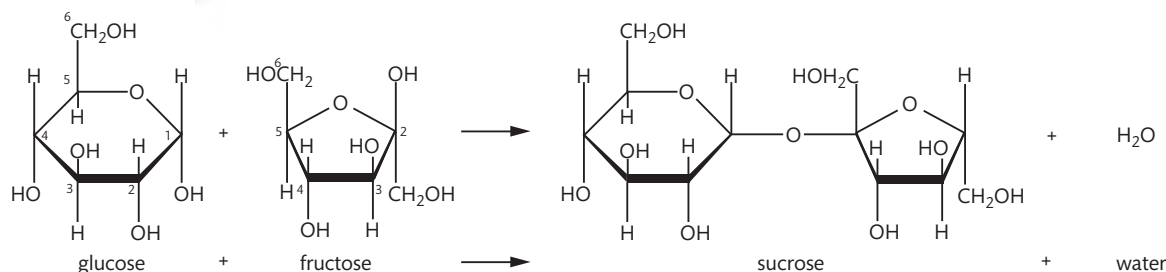
Humans and other animals have difficulty absorbing relatively large triglycerides and their digested form (fatty acids) from the intestine into the bloodstream. Chylomicrons are very small particles made up primarily of fat and some protein. Chylomicrons are produced in the alimentary canal and then transported into the bloodstream. They are used to transport fats to the liver and other tissues in the body. If your doctor orders a lipid blood test, chylomicrons are some of the low-density lipoproteins (LDL) that are measured. If their levels in the blood are elevated, they are referred to as the 'bad lipoproteins'.

Some textbooks will refer to condensation reactions as dehydration synthesis reactions. The names condensation and dehydration synthesis are both good reminders that water is always one of the products of these reactions.



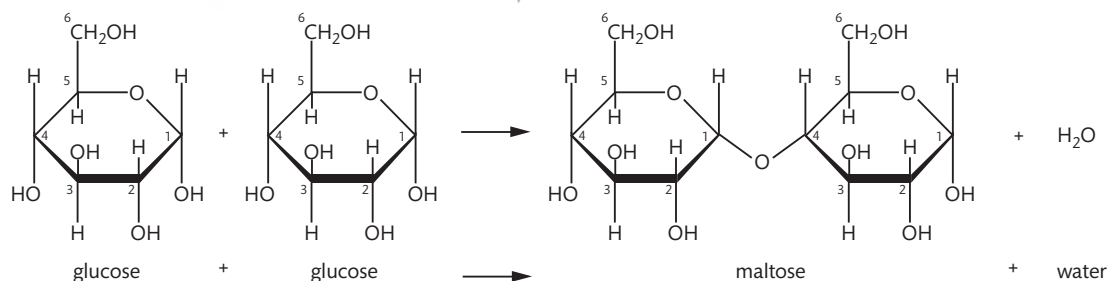
Let's look at a detailed example of a condensation reaction occurring between two monosaccharides. The example in Figure 2.13 shows the formation of the disaccharide sucrose from the reaction between the two monosaccharides glucose and fructose.

In similar reactions, other disaccharides are formed by different monosaccharides undergoing a condensation reaction. Figure 2.14 shows the condensation reaction that forms the disaccharide maltose from two alpha-D-glucose molecules. In a very similar way, the disaccharide lactose is formed by the condensation reaction between alpha-D-glucose and the monosaccharide galactose.



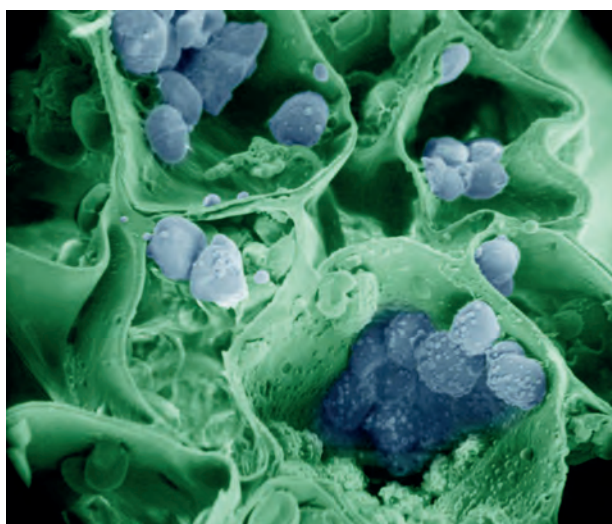
**Figure 2.13** The condensation reaction between glucose and fructose to form the disaccharide sucrose and a water molecule. Each corner of the sugar rings has an 'unshown' carbon atom. Each carbon atom is numbered in the reactants. Glucose and fructose are isomers of each other because they have the same chemical formula,  $C_6H_{12}O_6$ .

**Figure 2.14** A condensation reaction showing the formation of the disaccharide maltose. Notice that water is always a product of a condensation reaction and that one of the two monosaccharides 'donates' a hydroxide ion ( $^-OH$ ) and the other monosaccharide 'donates' a hydrogen ion ( $H^+$ ), which combine to form the water molecule. The bond that is freed up is used to form the covalent bond between the two monosaccharides. All condensation reactions occur in a very similar way.



## Monosaccharides: the building blocks of polysaccharides

Condensation reactions can be used to synthesize even larger molecules by accomplishing the same or a similar reaction on more than one area of a monomer such as a monosaccharide. Repeatedly bonding glucose monosaccharides produces a variety of very large molecules or polymers. Some examples are cellulose, starch, and glycogen; Table 2.5 summarizes their functions.



Scanning electron micrograph (SEM) of sliced open plant cells. The plant cell walls composed largely of cellulose are clearly visible, and in the interior of the cells are chloroplasts, which produce and store carbohydrates such as starch.

**Table 2.5 The functions of major polysaccharides**

Polysaccharide	Summary of functions
Cellulose	Major component of plant cell walls, helps give rigidity/support to plant parts such as roots, stems, and leaves
Starch	Organic products of photosynthesis are stored in plants as starch, typically as starch granules in chloroplasts or in plant storage areas such as roots or root structures
Glycogen	Animals store excess glucose in this form. Glycogen is stored in the liver and in muscle tissue

At the end of this section, use the hotlinks to view and manipulate three-dimensional models of cellulose, starch, and glycogen. When viewing these structures online, take note of the following.

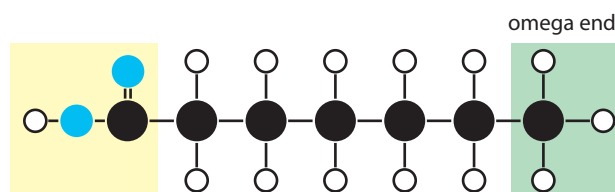
- Cellulose, starch, and glycogen are all polysaccharides of the same monomer unit, glucose.
- The bonding mentioned with each molecule, such as 1,4 linkages, refers to the carbon numbers of the glucose molecules that create the covalent bond.
- Starch has two subcomponents, amylopectin and amylose.
- Amylose is the only one of the three glucose polysaccharides that is a linear molecule with no side branching.
- All three polysaccharides can be composed of many thousands of glucose monomers.

## Fatty acids

Although they have similarities in their molecular structure, not all fatty acids are identical. All fatty acids have a carboxyl group ( $-\text{COOH}$ ) at one end and a methyl group ( $\text{CH}_3-$ ) at the other end. In between is a chain of hydrocarbons (hydrogen atoms and carbon atoms) that is usually between 11 and 23 carbons long (12–24 carbons when counting the carbon of the methyl group as well).



**Figure 2.15** The three sections found in all fatty acids: the carboxyl group at one end, the long hydrocarbon chain in the middle, and the methyl group at the other end. The end with the methyl group is also called the omega end.

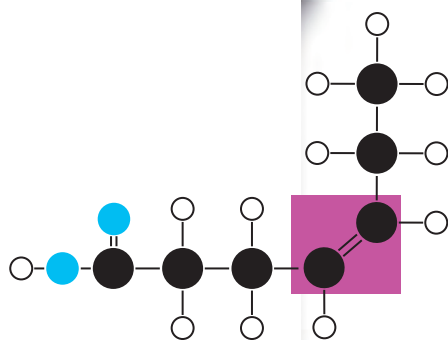


Saturated fatty acids are called that because the carbons are carrying as many hydrogen atoms as they can, in other words they are saturated with hydrogen atoms. These molecules are typically found in animal products such as butter, bacon, and the fat in red meat. These fats are generally solid at room temperature. Because the carbons are carrying as many hydrogen atoms as possible, saturated fatty acids have no double bonds between the carbon atoms. The shape of the molecule is straight: there are no kinks or bends along the chain.

### Monounsaturated fatty acids

If one double bond exists in the chain of hydrocarbons, the fatty acid is not saturated any longer: it has two empty spaces where hydrogen atoms could be. This type of unsaturated fatty acid is referred to as monounsaturated.

In Figure 2.16, the double bond between two carbons in the hydrocarbon chain is highlighted. Notice how the absence of two consecutive hydrogen atoms on the same side of the carbon atom chain causes the molecule to bend at the zone where the double bond is.



**Figure 2.16** The highlighted zone in the middle of the fatty acid shows that it has a single double bond in the hydrocarbon chain. This creates a bend or kink in the shape of the molecule. Note: Fatty acids typically have more carbons than the one shown for this illustration.

### Polyunsaturated fatty acids

Polyunsaturated fatty acids have at least two double bonds in the carbon chain. They typically come from plants (olive oil is an example). These fatty acids are called polyunsaturated because two or more carbons are not carrying the maximum number of hydrogen atoms (another way of saying this is that two or more carbons are double bonded to each other). Lipids that contain polyunsaturated fatty acids tend to be liquids at room temperature.

Imagine a hydrocarbon chain several times longer than any shown in the figures so far, with several more double bonds. The molecule may have so many bends/kinks that it starts to curve over onto itself or twist around itself. This frequently happens with polyunsaturated fatty acids.

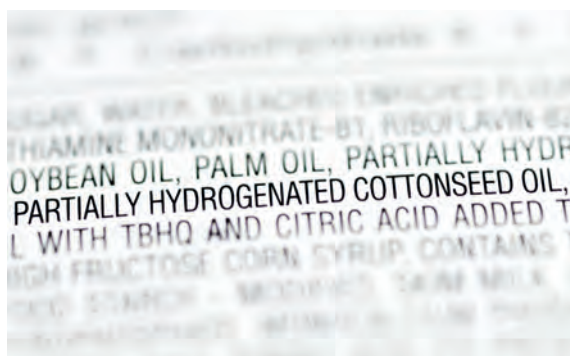
### Hydrogenation: *cis* and *trans* fatty acids

In many heavily processed foods, polyunsaturated fats are often hydrogenated or partially hydrogenated as part of the processing. This means the double bonds (and hence the kinks) are eliminated (or partly eliminated) by adding hydrogen atoms. Hydrogenation straightens out the natural bent shape of unsaturated fatty acids. Naturally curved fatty acids are called *cis* fatty acids, and the hydrogenated,

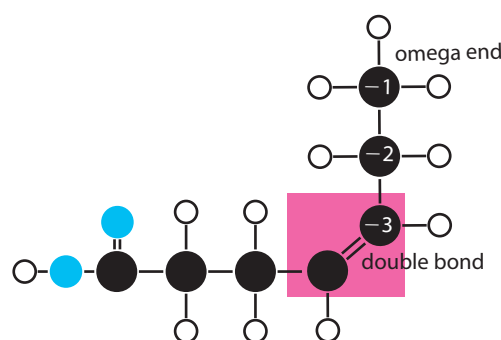
The calorie count based on lipid content is important for many people in order to maintain a healthy weight, but the type of lipids found in foods that gives those calories should be important to everyone.

straightened ones are called *trans* fatty acids. The vast majority of *trans* fatty acids are the result of chemical transformations in food-processing factories. They are usually only partially hydrogenated and thus still contain one or more double bonds.

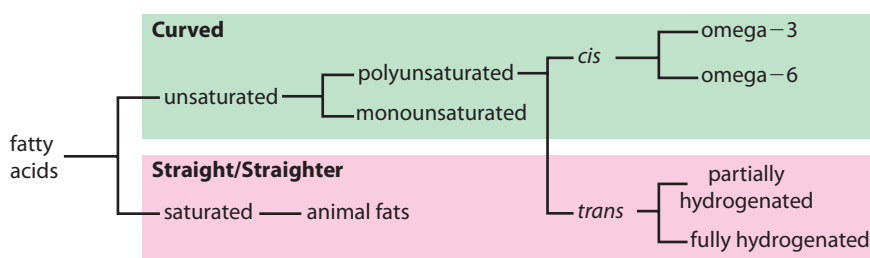
One category of *cis* fatty acids is called omega-3. The name comes from the fact that the first carbon double bond to be found in this molecule is at the third carbon atom counting backwards from the omega end (see Figure 12.17). Fish are a good source of omega-3 fats.



Part of the ingredients list of a bought cake. 'Partially hydrogenated' means that this is a product that contains *trans* fats.



**Figure 2.17** This sketch shows how the name omega-3 is derived for some fatty acids. Starting at the omega carbon, count the carbons until you reach the first double bond.



**Figure 2.18** Summary of fatty acid types.

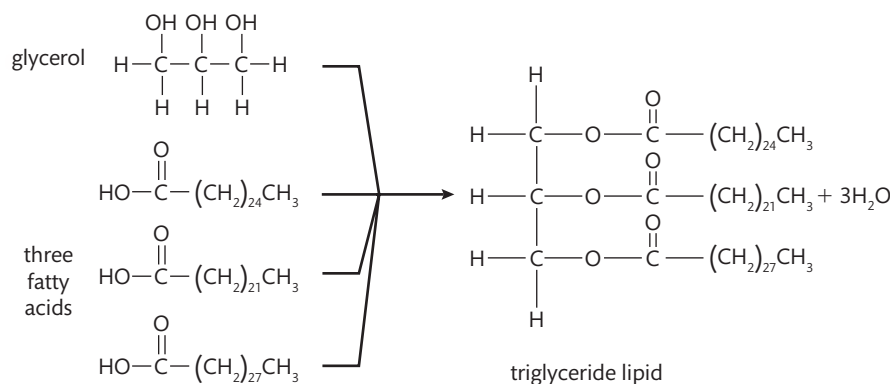
## Condensation reactions result in the formation of triglyceride lipids

The component molecules of triglyceride lipids (fats in animal cells and oils in plant cells) are glycerol and three fatty acids. The identity and thus characteristics of the three fatty acids in each triglyceride will determine the overall characteristics of the fat or oil. Triglycerides vary greatly from each other, including their relative healthiness in our diet. Figure 2.19 shows a representation of the condensation reaction that creates the covalent bonds between the glycerol portion and the three fatty acids of a triglyceride lipid. Notice that, as in all condensation reactions, a water molecule is created from each of the three reactions.



Condensation and hydrolysis reactions in biochemistry are so common that you will encounter information concerning those two types of reactions throughout your study of biology. Take the time to learn the basics of these two reaction types.

**Figure 2.19** Condensation reaction showing the four reactants necessary to form a triglyceride lipid. Notice that there are four products: the three water molecules as well as the triglyceride.



### NATURE OF SCIENCE

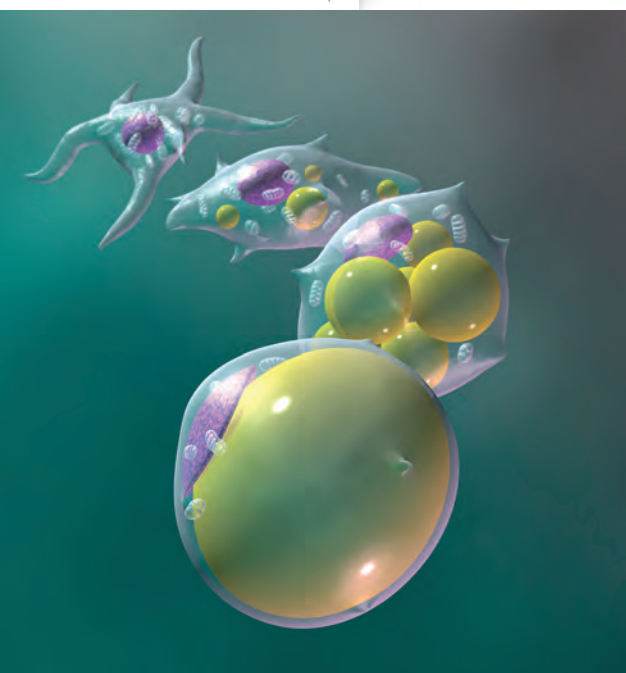
Now that you are familiar with the terminology and basic chemical structure of lipids and fatty acids, you are ready to read and evaluate some information concerning various types of lipids in your foods. Try to evaluate the information given by researching the following.

Use a search engine to research consumer information reported by food companies concerning lipids. Try:

- one or more of your favourite fast food restaurants (or at least some you know) and couple the restaurant name with 'nutrition information'
- the company and snack name of one or more of your favourite snacks plus 'nutrition information'
- other searches that you can think of that may or may not give you reasonably reliable information.

Diets characteristic of people in various areas of the world appear to have a huge influence on health and longevity.

This drawing shows a fat cell (adipocyte) becoming larger as lipids are stored in it.



## Energy storage solutions in humans

Humans and many other organisms have developed chemical strategies to store molecules in reserve to use for ATP production during the process of cell respiration. These include:

- storing glucose as the polysaccharide glycogen in liver and muscle tissues
- storing triglyceride lipids within adipose (fat) cells.

Triglyceride lipids, when needed, can be hydrolysed into two carbon segments that can enter into cell respiration at a chemical sequence point that is very efficient for the production of ATP. Thus lipids have about twice the energy content per gramme compared with other molecules, such as carbohydrates and proteins, that are also used for cell respiration.

Lipids have another advantage as a long-term energy storage molecule: they are insoluble in water (such as in the aqueous environments of cytoplasm, intercellular fluid, and blood plasma), and so they do not upset the osmotic balance of solutions. If humans were to store large concentrations of glucose in certain cells of the body for long-term energy storage, those cells would swell to ridiculous proportions because the glucose would attract water into the cells due to the surrounding hypotonic fluids.

## Calculating the body mass index

The use of an indexed value known as the body mass index (BMI) as an indicator of healthy weight has recently become popular. The BMI is a number that reflects both the weight and the height of a person. The idea is that people who are taller should weigh more. There are three ways that you can determine your BMI:

- using a formula, based on either metric or imperial measurements of weight and height
- using a graph known as a nomogram to read the BMI value from a central intersection point between weight and height measurements
- using an online calculator that outputs the BMI after the height and weight measurements have been input.

Each of the methods used to determine the BMI must be correlated with information concerning the BMI that shows whether a value reflects someone being underweight, normal in weight, overweight, or obese. Such charts often come with a caution that states children and pregnant women should not use them. Table 2.6 shows the data provided by the Centers for Disease Control and Prevention (CDC).

**Table 2.6 Interpreting BMI values**

BMI	Description category
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0 and above	Obese

Here are the two formulas for calculating BMI:

- formula 1, metric units,  $\text{BMI} = \text{weight (kg)} / [\text{height (m)} \times \text{height (m)}]$
- formula 2, imperial units,  $\text{BMI} = \text{weight (lb)} / [\text{height (in)} \times \text{height (in)}] \times 703$

Example 1 (metric): for someone who is 1.70 m and weighs 58 kg, his or her  $\text{BMI} = 58 / (1.7 \times 1.7) = 20.1$ . Therefore this person is categorized as having a normal weight.

Example 2 (imperial): for someone who is 5'10" (5'10" = 70") and weighs 235 lb, his or her  $\text{BMI} = 235 / (70 \times 70) \times 703 = 33.7$ . This person is categorized as obese.



Colorized magnetic resonance image (MRI) of a woman with a very high BMI. Among a myriad of other possible problems, the extra body mass present in obese patients puts a strain on their heart and lungs.



Some, but not all, countries make a concerted effort to inform their citizens of the health risks and benefits of certain foods/diets. This is why good scientific research on the consequences and benefits of certain food types is essential.



To learn more about the three-dimensional models of cellulose, starch, and glycogen, and calculating BMI, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.3.



#### NATURE OF SCIENCE

Looking for patterns, trends, and discrepancies: most but not all organisms assemble proteins from the same amino acids.



### CHALLENGE YOURSELF

- 3 Calculate the BMI of a person who is 1.64 m tall and weighs 79 kg. Using Table 2.6, which category would be used to describe him or her?
  - 4 Calculate your own BMI after measuring your height and current weight.
- At the end of this section, use the hotlinks to go to a website that includes a nomogram and online calculator for determining BMI.
- 5 Use the online calculator to confirm your own BMI calculation.
  - 6 Use the nomogram to confirm your own BMI calculation.

### Exercises

- 6 Write the word equation for the condensation reactions that would produce a triglyceride lipid from its four molecular subcomponents.
- 7 Rank these fatty acids types from the least to the most healthy: saturated fatty acid; unsaturated fatty acid; *trans* fatty acid.
- 8 Why is BMI a better reflection of a person's health compared with body mass alone?

## 2.4 Proteins

### Understandings:

- Amino acids are linked together by condensation to form polypeptides.
- There are 20 different amino acids in polypeptides synthesized on ribosomes.
- Amino acids can be linked together in any sequence, giving a huge range of possible polypeptides.
- The amino acid sequence of polypeptides is coded for by genes.
- A protein may consist of a single polypeptide or more than one polypeptide linked together.
- The amino acid sequence determines the three-dimensional conformation of a protein.
- Living organisms synthesize many different proteins with a wide range of functions.
- Every individual has a unique proteome.

### Applications and skills:

- Application: Rubisco, insulin, immunoglobulins, rhodopsin, collagen, and spider silk as examples of the range of protein functions.
- Application: Denaturation of proteins by heat or by deviation of pH from the optimum.
- Skill: Drawing molecular diagrams to show the formation of a peptide bond.

#### Guidance

- The detailed structure of the six proteins selected to illustrate the functions of proteins is not needed.
- Egg white or albumin solutions can be used in denaturation experiments.
- Students should know that most organisms use the same 20 amino acids in the same genetic code, although there are some exceptions. Specific examples could be used for illustration.

### Formation of polypeptides

Cells use the naturally occurring 20 amino acids to synthesize polypeptides. They do this under the control of DNA, each polypeptide being created under the control of a specific area of a specific DNA molecule called a gene. In a multicellular organism, every cell of that organism has the same set of chromosomes and thus the same DNA. Each cell that has differentiated to have a specific function in a specific tissue of the body only uses the genes that are necessary for that cell type. Some of those genes are

almost universal, such as the genes that code for proteins involved in common cell functions. A good example of this would be the protein components that make up ribosomes, as all cells need ribosomes. In addition, each specific cell type then uses the genes that help accomplish the specific activities necessary for that cell type. A cell of the human pancreas would ‘turn on’ the gene for synthesis of the peptide hormone insulin, whereas most cells would not activate that gene even though the gene is present in all human cells. The total number of (possibly) active genes in any living organism is difficult to determine with accuracy. A current estimate for human beings is somewhere between 20 000 and 25 000 genes in each of our cells. This is nothing to brag about though, as a high gene count falls somewhere between grape plants (which have about 30 000 genes) and chickens (which have about 17 000 genes). This shows why it would be a mistake to correlate the number of genes with organism complexity. Table 2.7 shows a selection of organisms and their approximate gene count.

**Table 2.7 Selected organisms and their approximate number of genes**

Common name of the organism	Approximate number of genes in the organism’s genome
Yeast (single-celled fungi)	6 000
<i>Drosophila</i> (fruit fly)	14 000
Rice plant	51 000
Laboratory mouse	30 000
Domestic dog	19 000
Humans	20–25 000

No matter how many genes an organism has within its genome, all genes are the genetic code for the possible polypeptides found within that organism, and all polypeptides are synthesized from the same monomers, specifically amino acids. Although there are a few exceptions to this, virtually all organisms use the same genetic code, and they use the same 20 amino acids to construct their polypeptides.

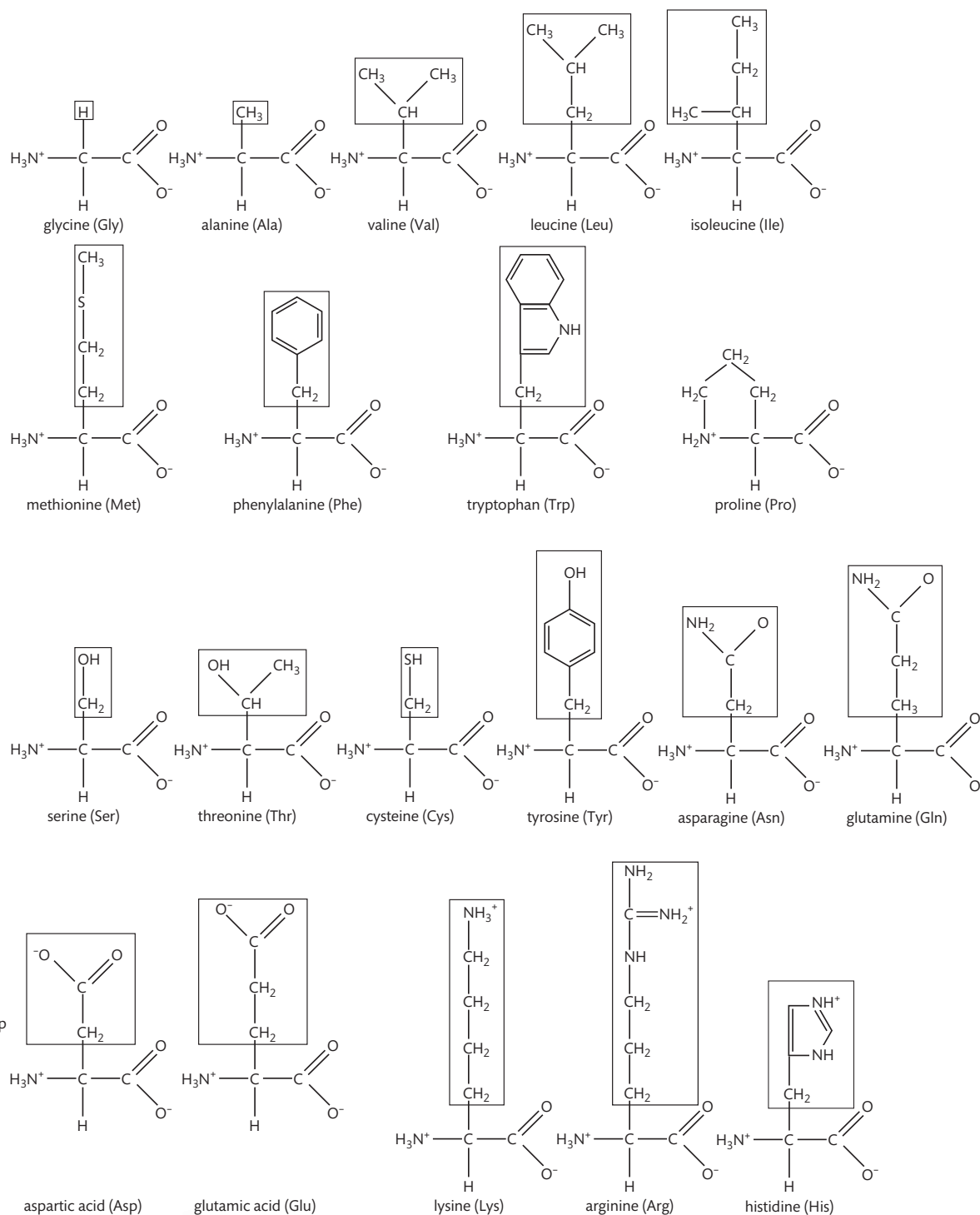
Each of the 20 amino acids differs from the others in one bonding location around the central carbon atom; that difference in structure is called the R or variable group of the amino acid (Figure 2.20). You do not need to memorize the R-groups but you do need to memorize the general structure that applies to all amino acids.

When amino acids are in an aqueous solution, such as cytoplasm or blood plasma, the amine and carboxyl functional groups ionize, as shown in Figure 2.20. This ionization does not alter the covalent bonding pattern but it does make the functional groups look a little different as each carboxyl group has ‘lost’ a hydrogen ion and each amine group has gained a hydrogen ion.

When polypeptides are synthesized at ribosomes under the control of genes, the reaction that is occurring is a condensation reaction. The sequence of the amino acids is determined precisely by the DNA, but the condensation reactions are virtually identical.

## Polypeptides are highly variable

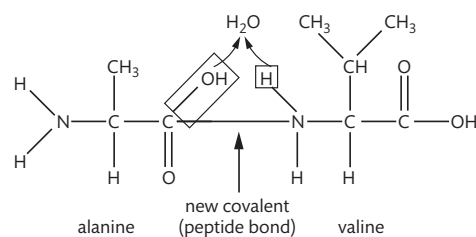
The condensation reactions described above do not occur between any two amino acids randomly. The order of the amino acids is always determined by triplets of nucleotides



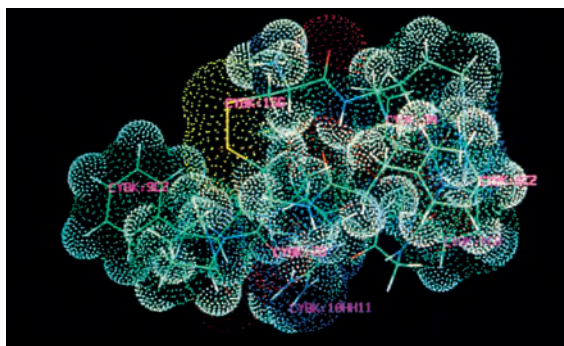
**Figure 2.20** A chart showing the structures of the 20 amino acids. The boxed areas shown are the R-groups of the amino acids. Note how each amino acid is identical except for the variable R-group.

**Figure 2.21** Condensation reaction between the amino acids alanine and valine.

Note that for simplicity the amine and carboxyl groups are being shown in a non-ionized form. This reaction looks the same for any two amino acids, as the only change would be to the R (variable) groups.



along nucleic acid molecules (DNA and RNA), and is directed by a ribosome. As there are 20 amino acids, there is a large choice for the sequence of the amino acids as well as the total number of amino acids to use within a polypeptide. Each polypeptide that has been selected for a specific purpose has not only its own amino acid sequence, but also its own three-dimensional shape; that shape has a dominant influence on the function of the polypeptide. Even a change in a single amino acid in the overall sequence of a polypeptide can have drastic effects on its function.



## Levels of polypeptide and protein structure

Proteins serve a tremendous variety of functions in cells and organisms; Table 2.8 shows you just a few examples.

**Table 2.8 Some examples of proteins and their functions**

Rubisco	The short-hand name for the enzyme that catalyses the first reaction of the carbon-fixing reactions of photosynthesis
Insulin	A protein hormone produced by the pancreas that results in a decrease of blood sugar levels and an increase of sugar inside body cells
Immunoglobulin	Another name for an antibody that recognizes an antigen(s) as part of the immune response
Rhodopsin	A pigment found in the retina of the eye that is particularly useful in low light conditions
Collagen	The main protein component of connective tissue, which is abundant in skin, tendons, and ligaments
Spider silk	A fibrous protein spun by spiders for making webs, drop lines, nest building, and other uses

Given the myriad of functions of proteins, they have to be capable of assuming many forms and structures. The function of any particular protein is closely related to its structure. There are four levels of organization to protein structure: primary, secondary, tertiary, and quaternary.

- Primary protein structure: the sequence of amino acids within the protein; this sequence determines the three-dimensional shape, as shown below.
- Secondary protein structure: repetitive shapes of either a helix (a spiral staircase shape) or a pleated sheet (a sheet with corrugated folds), e.g. spider silk.
- Tertiary structure: a shape often described as globular, e.g. enzymes.
- Quaternary: two or more polypeptides combined together to make a single functional protein, e.g. haemoglobin.

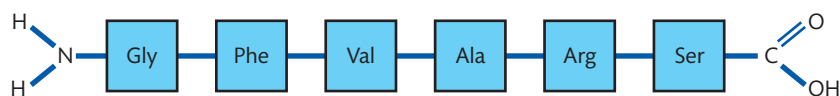
## CHALLENGE YOURSELF

- 7 Use the amino acid structures in Figure 2.20 and draw the following short peptide consisting of five amino acids. The *n*-terminal end begins with an amine group and the *c*-terminal end finishes with a carboxyl group.  
*n*-terminal end [ Valine – Glycine – Serine – Threonine – Alanine ] *c*-terminal end

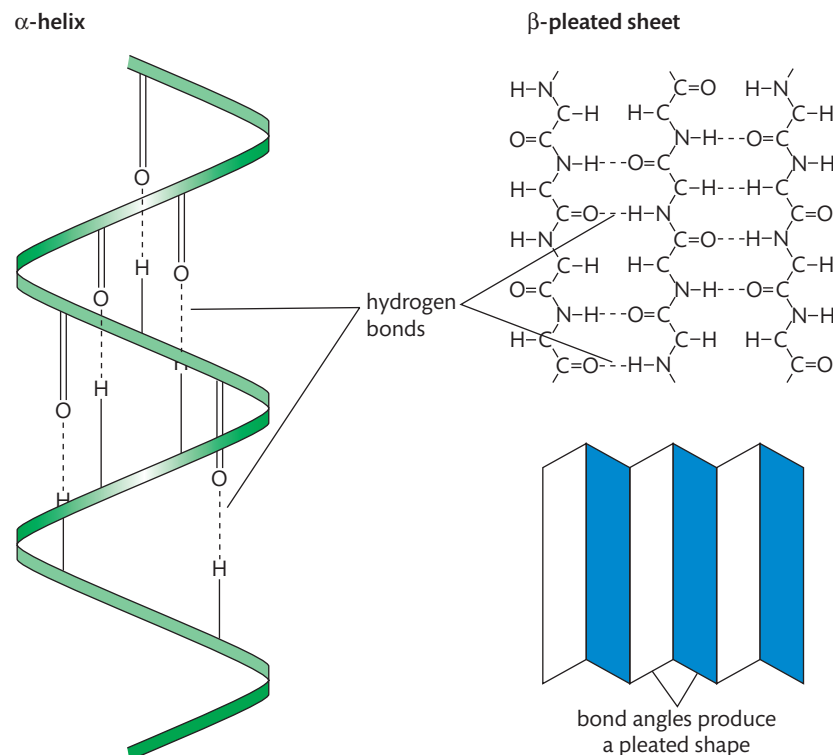
Computer graphic representation of the structure of bradykinin, a polypeptide that is active in human metabolism. Despite the apparent complexity, this is a relatively short peptide consisting of only nine amino acids.



**Figure 2.22** Simplistic example of a polypeptide's primary structure.



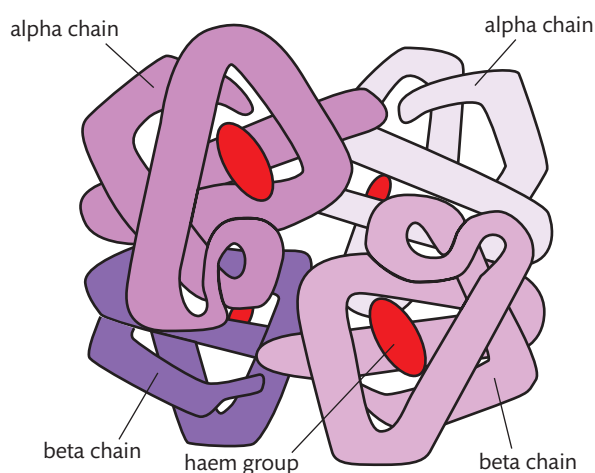
**Figure 2.23** The two geometric patterns of protein secondary structures. The variable or R-groups are not shown in secondary structures as they are not involved in creating the molecular shape.



When trying to identify individual amino acids within a large, complex polypeptide, try to identify the peptide bonds between each of the covalently bonded amino acids. That bond will always be a nitrogen atom bonded to a carbon atom, with that carbon atom also doubled bonded to an oxygen.



**Figure 2.24** Molecular model of the protein structure of haemoglobin. Each haemoglobin molecule is considered to be a single protein. Each contains four polypeptide chains held together in a quaternary structure. Some of the same types of bonds important for creating the tertiary structure also help to hold quaternary structure proteins together.



## Some proteins are more than one polypeptide

Frequently, the terms polypeptide and protein are used interchangeably. In fact, based on the biochemistry of proteins, the two terms do have a slightly different meaning. A protein is an organic substance consisting of covalently bonded amino acids, and it is ready to carry out its function. If the protein is an enzyme, it is ready to catalyse

a reaction. If the protein is an antibody (immunoglobulin), it is ready to bond to an antigen as part of an immune response. The point is, a protein is able to carry out its intended function. That may or may not be true for a polypeptide.

A polypeptide is a single amino acid chain with its own primary structure. It has a single *c*-terminal end and a single *n*-terminal end. If the single polypeptide is able to carry out its function as it is, then that polypeptide is considered to be a protein.

Some polypeptides cannot serve a biochemical function until they combine with one or more other polypeptide(s). If you recall, this is what is called a quaternary structure. When two or more polypeptides bond together and then are ready to accomplish their function, together they are considered to be a single protein.

## Your unique proteome

Over the last few decades we have come to know that each individual organism of a species is genetically different from all other organisms. This is especially true for organisms that reproduce by sexual reproduction. The specific DNA sequence that is unique to one individual is called a genome. As DNA is the genetic code for proteins, this means that each individual has a unique set of proteins that he or she is capable of synthesizing. Thus each individual is said to have a unique proteome as well as a unique genome.

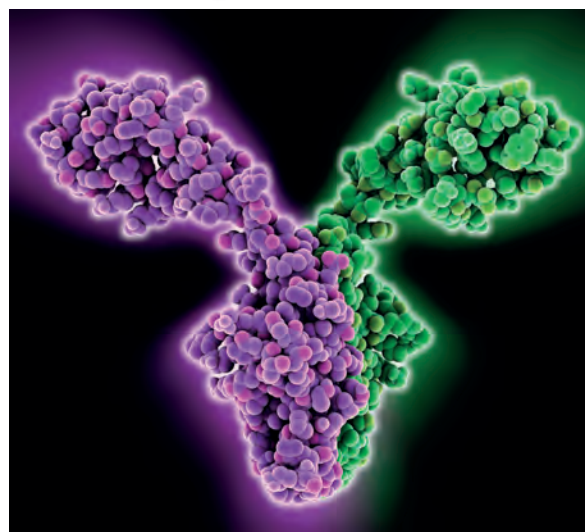
## Proteins can be denatured by heat and alteration of the pH environment

The intra-molecular bonds of proteins that hold together their secondary, tertiary, and quaternary structures are susceptible to alterations in normal temperature and pH; the intra-molecular bonds can be disrupted. When a protein takes on a three-dimensional shape, it does this because of the interactions of the amino acids with each other.

When protein molecules are placed into a temperature environment that is higher than their physiological optimum, the increased molecular motion puts a great deal of stress on many of the relatively weak intra-molecular bonds. This can result in the primary structure remaining intact (the sequence of amino acids connected by peptide bonds) but the hydrogen bonds often cannot stay in place under the stress caused by the increased molecular motion. The result is that the protein loses its normal three-dimensional shape and function. A protein's function is directly dependent on its shape; in most instances, as long as the covalent bonds (like peptide bonds) remain intact, the protein will return to its normal shape and function if it is returned to its normal temperature.

A similar phenomenon occurs when a protein is placed in a pH environment that is not close to its optimum pH. A protein will lose its normal three-dimensional shape, and thus lose its functionality, in these circumstances. When a fluid environment such as cytoplasm, blood plasma, etc., is flooded with either  $H^+$  ions (an acid) or  $OH^-$  ions (a base), the extra charges can prevent normal hydrogen bonding. Thus the protein will not take on its 'normal' shape and will not function normally.

This is a protein found in the ribosomes of some bacteria. The computer model of the protein clearly shows that the protein is composed of two polypeptides. Each of the polypeptides would require a different gene within the bacteria's genome to code for its synthesis.



For centuries people have been selectively breeding both crops and animals to increase their food value. Recently, some companies have begun genetically modifying foods using biotechnology. The jury is still out regarding whether this approach will ultimately be both beneficial and safe.



**Technically, a proteome is the collection of proteins found within a particular cell type at a specified time under a specific set of environmental circumstances. Cells in multicellular organisms differentiate and thus do not produce the same proteins even though they contain the same genome.**



Some living organisms have evolved proteins and other molecules that remain stable and functional at very high temperatures. This is a hot spring called Morning Glory in Yellowstone National Park, USA. The brilliant colours you see in the water are primarily the result of the growth of cyanobacteria that can live in water temperatures as high as 165°C.

### Exercises

- 9 Study the amino acid chart (Figure 2.20) and find the amino acids that meet the following criteria.
  - (a) The single amino acid whose non-R-group shape is slightly different compared with all the others.
  - (b) The two amino acids that contain sulfur atoms.
  - (c) The five amino acids that contain either a carboxyl or an amine group as part of their R-group.
- 10 How many peptide bonds would be found in a polypeptide that contains 76 amino acids?
- 11 Considering only the usual 20 naturally occurring amino acids, how many combinations of amino acids would be possible if four amino acids were to bond together in a random order?



### NATURE OF SCIENCE

Most, but not all, organisms assemble proteins from the same 20 amino acids. Virtually every reference concerning amino acids will tell you that there are 20 amino acids in nature. It is true that the universal genetic code (universal indicating that it is used in the vast majority of organisms on Earth) only encodes 20. But in nature there are frequently exceptions, and that includes things that are called 'universal'. If you include all known living organisms then there are 22 amino acids that are used to create polypeptides. In addition to the 20 amino acids whose structures are given in Figure 2.20, there are two additional amino acids called selenocysteine and pyrrolysine.

## 2.5 Enzymes

### Understandings:

- Enzymes have an active site to which specific substrates bind.
- Enzyme catalysis involves molecular motion and the collision of substrates with the active site.
- Temperature, pH, and substrate concentration affect the rate of activity of enzymes.
- Enzymes can be denatured.
- Immobilized enzymes are widely used in industry.

### Applications and skills:

- Application: Methods of production of lactose-free milk and its advantages.
- Skill: Design of experiments to test the effect of temperature, pH, and substrate concentration on the activity of enzymes.
- Skill: Experimental investigation of a factor affecting enzyme activity.

#### Guidance

- Lactase can be immobilized in alginate beads, and experiments can then be carried out in which the lactose in milk is hydrolysed.
- Students should be able to sketch graphs to show the expected effects of temperature, pH, and substrate concentration on the activity of enzymes. They should be able to explain the patterns or trends in these graphs.

### NATURE OF SCIENCE

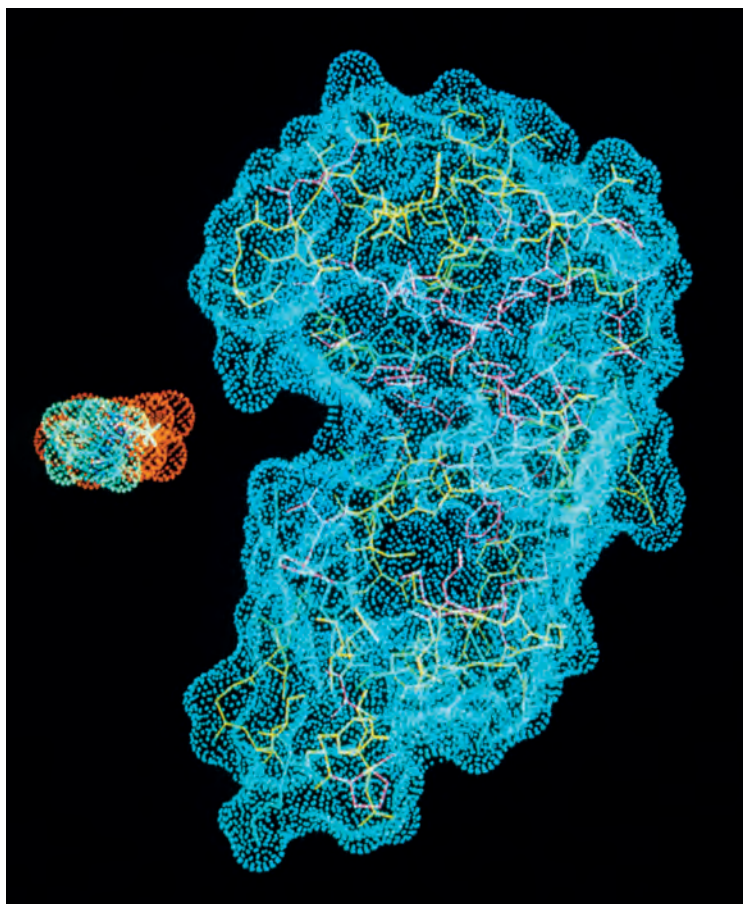
Experimental design: accurate, quantitative measurements in enzyme experiments require replicates to ensure reliability.



## Enzymes are organic molecules that act as catalysts

Enzymes are proteins. Thus enzymes are long chains of amino acids that have taken on a very specific three-dimensional shape. Think of a flexible metal wire that can be

bent many times into what is called a globular shape. This shape is complex and at first glance appears to be random, but in enzymes (and other globular proteins) the complex shape is not random: it is very specific. Somewhere in the three-dimensional shape of the enzyme is an area that is designed to match a specific molecule known as that enzyme's substrate. This area of the enzyme is called the active site. The active site of an enzyme matches the substrate in a similar way to a glove fitting a hand. In this analogy, the glove represents the active site and the hand represents the substrate.



This computer graphic shows an enzyme (the larger molecule on the right) and its substrate. Notice the active site on the left-hand side of the enzyme.

Another analogy that is very commonly used for enzyme–substrate activity is a lock and key. In this analogy, the lock represents the enzyme's active site and the key represents the substrate. Because the three-dimensional shape of the internal portion of the lock is complex and specific, only one key will fit. The same principle is generally true for enzymes and their substrates: they are specific for each other.

It is not enough for an enzyme's substrate(s) to just enter an active site. The substrate(s) must enter with a minimum rate of motion that will provide the energy necessary for the reaction to occur. Enzymes do not provide this energy, they simply lower the energy minimum that is required. The energy being referred to is called the activation energy of the reaction. Thus enzymes lower the activation energy of reactions. Enzymes are not considered to be reactants and are not used up in the reaction. An enzyme can function as a catalyst many, many times. In addition, an enzyme cannot force a reaction to occur that would not otherwise happen without the enzyme; however, the reaction may be much more likely to occur with an enzyme because the input of energy (activation energy) required will be lower with the enzyme present.

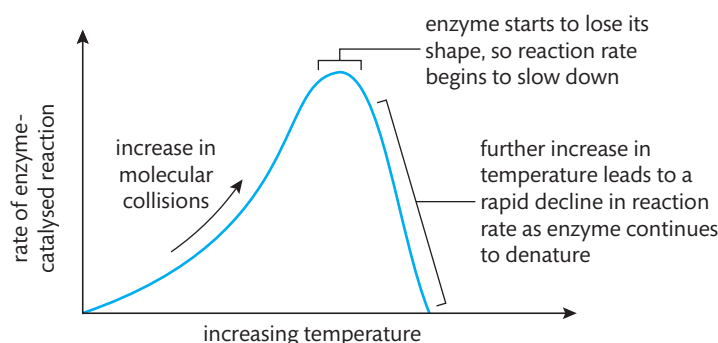


## Factors affecting enzyme-catalysed reactions

When you are considering the various environmental factors that affect enzyme-catalysed reactions, you must first remember that all chemical reactions are fundamentally molecules colliding. If the molecules that are colliding do so at a high enough rate of speed and the molecules have the capability of reacting with each other, then there is a chance that a reaction will occur. Enzymes cannot change those fundamentals.

### Effect of temperature

Imagine an enzyme and its substrate floating freely in a fluid environment. Both the enzyme and substrate are in motion and the rate of that motion is dependent on the temperature of the fluid. Fluids with higher temperatures will have faster moving molecules (more kinetic energy). Reactions are dependent on molecular collisions and, as a general rule, the faster molecules are moving, the more often they collide, and with greater energy. Reactions with or without enzymes will increase their reaction rate as the temperature (and thus molecular motion) increases. Reactions that use enzymes do have an upper limit, however (see Figure 2.25). That limit is based on the temperature at which the enzyme (as a protein) begins to lose its three-dimensional shape because the intra-molecular bonds are being stressed and broken. When an enzyme loses its shape, including the shape of the active site, it is said to be denatured. Denaturation is frequently temporary, as in many instances the intra-molecular bonds will re-establish when the temperature returns to a suitable level.



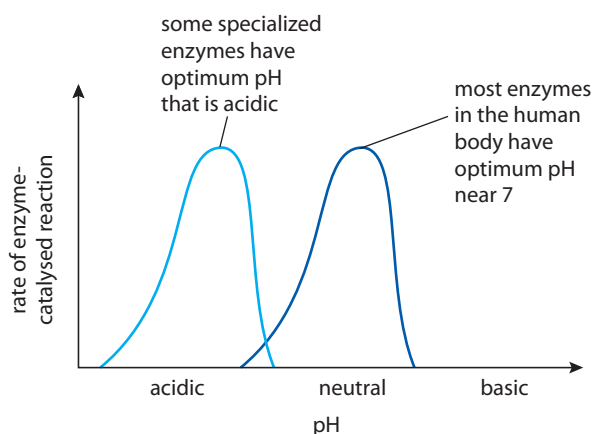
**Figure 2.25** The effect of increasing temperature on the rate of an enzyme-catalysed reaction.

Whether or not an enzyme is permanently destroyed by denaturation is largely dependent on whether covalent bonds (such as peptide bonds) have broken. DNA determines the order of amino acids, and they have no way of reassembling properly if they become detached from each other.

### Effect of pH

The active site of an enzyme typically includes many amino acids of that protein. Some amino acids have areas that are charged either positively or negatively. The negative and positive areas of a substrate must match the opposite charge when the substrate is in the active site of an enzyme, in order for the enzyme to have catalytic action. When a solution has become too acidic, the relatively large number of hydrogen ions ( $H^+$ ) can bond with the negative charges of the enzyme or substrate, and prevent proper charge matching between the two. A similar scenario occurs when a solution has become too basic: the relatively large number of hydroxide ions ( $OH^-$ ) can bond with the positive charges of the substrate or enzyme, and once again prevent proper charge matching between the two. Either of these scenarios will result in an enzyme becoming less efficient, and in extreme situations becoming completely inactive. One further possibility is that the numerous extra positive and negative charges of acidic and basic solutions can result in the enzyme losing its shape and thus becoming denatured.

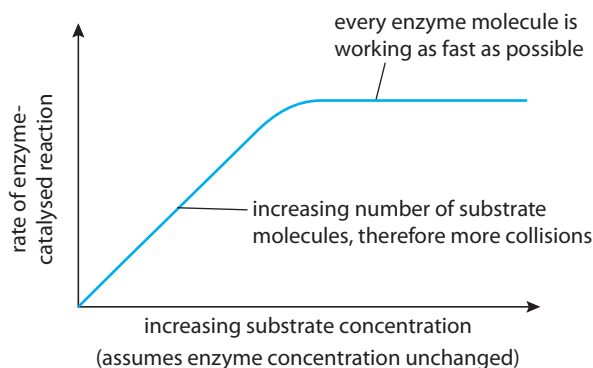
There is no one pH that is best for all enzymes (see Figure 2.26). Many of the enzymes active in the human body are most active when in an environment that is near neutral. There are exceptions to this, however; for example, pepsin is an enzyme that is active in the stomach. The environment of the stomach is highly acidic and pepsin is most active in an acidic pH.



**Figure 2.26** The effect of pH on the rate of an enzyme-catalysed reaction. This illustrates that there is no single pH that is best for all enzymes.

### Effect of substrate concentration

If there is a constant amount of enzyme, as the concentration of a substrate increases, the rate of reaction will increase as well (see Figure 2.28). This is explained by the idea of increased molecular collisions. If you have more reactant molecules, there are more to collide. There is a limit to this, however, because enzymes have a maximum rate at which they can work. If every enzyme molecule is working as fast as possible, adding more substrate to the solution will not increase the reaction rate further (see Figure 2.28).



**Figure 2.28** The effect of increasing the substrate concentration on the rate of an enzyme-catalysed reaction.

### Use of immobilized enzymes in industry

Cells are not the only 'factories' that make good use of enzymes. In the last 50 years, many industrial applications have been developed that make use of these catalytic proteins. However, there are major problems that have to be overcome. For example, if you want to catalyse one particular reaction, you need a pure enzyme, not a mixture as found in cells. Extracting or producing pure enzymes in the large quantities needed for industrial use is expensive. Because of their cost, enzymes in industry need to be reused repeatedly. The problem is that it is difficult to remove enzymes from liquid products in solutions so that the enzymes can be used further. One answer to this

	pH
strongly acidic	1
	2
	3
	4
weakly acidic	5
	6
neutral	7
weakly alkaline	8
	9
strongly alkaline	10
	11
	12
	13
	14

**Figure 2.27** The pH scale. Most fluids within the human body are close to neutral. The pH of blood plasma is typically 7.4, making it very slightly alkaline.

The pH scale is a logarithmic scale. This means that each whole number on the pH scale represents an increase or decrease by a power of 10. Thus a solution with a pH of 4 has 10 times more relative hydrogen ions compared with a solution with a pH of 5. That same solution with a pH of 4 has 100 times more relative hydrogen ions compared with a solution with a pH of 6.

problem is to invent ways to trap the enzymes in place and prevent them from getting washed out with the products. Researchers found that an enzyme could be held in place in tiny pores on beads of a substance called calcium alginate. Those enzymes trapped in the pores are said to be immobilized. As long as the alginate beads are recovered in the industrial process, the enzymes are also recovered and can be reused.

### Use of immobilized lactase to produce lactose-free milk

The majority of humans are born with the ability to digest lactose, one of the most common sugars found in milk. The reason for this is that we are born with the ability to produce the enzyme lactase in our digestive tract. Lactase is the enzyme that digests the disaccharide lactose into two monosaccharides (glucose and galactose); the monosaccharides are much more readily absorbed into the bloodstream. Most people lose the ability to produce lactase as they get older, and by adulthood no longer produce any significant amount of lactase. These people are said to be lactose intolerant. Normal milk and milk products enter their digestive tract and are not digested; instead the normal bacterial colonies in their intestines feed directly on the lactose. In effect, these bacterial colonies are being overfed. This leads to symptoms such as cramping, excessive gas, and diarrhoea.

In order to avoid these unpleasant symptoms, people who are lactose intolerant can eat milk and milk products that have been treated with lactase before consumption. With this treatment, the nutrients in the milk are not affected but the disaccharide lactose has been pre-digested, so a lactose-intolerant person is able to absorb the monosaccharide sugars.

One of the ways to pre-digest milk products on a large industrial scale is to use the method described above. Specifically, lactase enzyme molecules are trapped in the small pores of alginate beads and then milk and milk products are exposed to these beads for enough time for pre-digestion to occur.

It has been found that there is an extremely high incidence of lactose intolerance in some ethnic groups and a relatively low incidence in others. This is a good example of natural variation in a population.

There are more people with lactose intolerance than there are people who do not have the condition. In genetics, lactose intolerance is called the wild-type (the most common phenotype in a natural population).

### Investigation of factors affecting enzyme activity

*\*Safety alerts: Eye protection and lab aprons should be worn for all stages of these experiments.\**

Enzymes are protein catalysts. The catalytic ability of an enzyme can be optimized in certain pH and temperature environments, as well as by increasing the substrate concentration available to the enzyme. Because enzymes are proteins, they are subject to the same denaturing factors that affect all other proteins, including pH environments that are far from their optimum, and temperature environments that put stress on their internal bonds that help shape the molecule.

Note: This lab is designed for a class to be divided into three groups, each assigned one of the following questions.

- 1 What is the effect of altering the pH environment on the activity of the enzyme lactase? Hypothesis for question 1: the optimum pH environment for lactase will be slightly acidic (pH 6.0–6.5).
  - 2 What is the effect of altering the temperature environment on the activity of the enzyme lactase? Hypothesis for question 2: the optimum temperature environment for lactase will be 25°C.
  - 3 What is the effect of altering the concentration of substrate (lactose) on the activity of the enzyme lactase? Hypothesis for question 3: the optimum substrate concentration for lactase will be a ratio of 20 parts lactose by mass to 1 part lactase by mass.
- The following locally available reagents will need to be purchased: lactose powder (available from food shops), lactase powder or tablets, and glucose test strips (available from pharmacies). An alternative to using glucose test strips is to use Benedict's reagent, following standard protocols. An alternative for lactose powder is milk; use powdered milk if you want to compare the ratio of lactose mass to lactase mass, as in question 3.

- In addition, pH strips or another means of measuring the pH of solutions will be needed for the pH group, as well as buffered solutions for the desired pH. Bulb thermometers will be needed for the temperature group, and a mass scale for the substrate concentration group.
- Standard glassware and supplies, such as stirring rods, spatulas, test tubes, beakers, etc., will also be needed, based on your chosen techniques for carrying out the tests.

**\*Safety alerts: Eye protection and lab aprons should be worn for all stages of these experiments\***

- To make the enzyme solution (lactase), crush and add one lactase tablet to 200 ml water. Stir well until completely dissolved.
- To make the substrate solution (lactose), starting with powdered milk, follow the instructions given with the powder, and then decant the volumes needed.
- To carry out a negative control test (one that is designed to purposely give negative results), test the lactose solution using either a glucose test strip or Benedict's reagent (to show the absence of glucose).
- To carry out a positive control test (one that is designed to purposely give positive results), in a test tube add 2 ml of liquid milk and 1 ml of enzyme solution. Immediately mix well and start a timer. Test the solution for the presence of glucose after each 1-minute time period until the test is positive for glucose. Record the time necessary to achieve this positive result.

Each group will need to use the above standard procedures to design and carry out their own investigation by altering the solution pH, solution temperature, or the ratio of the mass of substrate to mass of enzyme (this mass ratio investigation should be based on the mass of the substrate and enzyme when in powder/tablet form). The dependent variable in each investigation will be the time necessary to achieve a positive glucose test.

Commercially available lactase has been formulated to still be active in the stomach and so is not sensitive to alterations in various acidic pH environments. Thus this investigation should attempt to start at a slightly acidic pH and have various increments to (safe) alkaline solutions.

Commercially available lactase is also quite temperature tolerant and will not completely denature until boiled for about 30 minutes.

## Exercises

- 12 Briefly explain why enzymes and substrates are specific for each other.
- 13 Why are enzymes considered to be catalysts of reactions?
- 14 How much more acidic is a solution of pH 3 compared with a solution of pH 6?



To learn more about enzymes, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2.5

## 2.6 Structure of DNA and RNA

### Understandings:

- The nucleic acids DNA and RNA are polymers of nucleotides.
- DNA differs from RNA in the number of strands present, the base composition, and the type of pentose.
- DNA is a double helix made of two antiparallel strands linked by hydrogen bonding between complementary base pairs.

### Applications and skills:

- Application: Crick and Watson's elucidation of the structure of DNA using model making.
- Skill: Drawing simple diagrams of the structure of single nucleotides of DNA and RNA, using circles, pentagons, and rectangles to represent phosphates, pentoses, and bases.

#### Guidance

- In diagrams of DNA structure, the helical shape does not need to be shown, but the two strands should be shown antiparallel. Adenine should be shown paired with thymine, and guanine with cytosine, but the relative lengths of the purine and pyrimidine bases do not need to be recalled, nor the numbers of hydrogen bonds between the base pairs.



### NATURE OF SCIENCE

Using models as representations of the real world: Crick and Watson used model making to discover the structure of DNA.



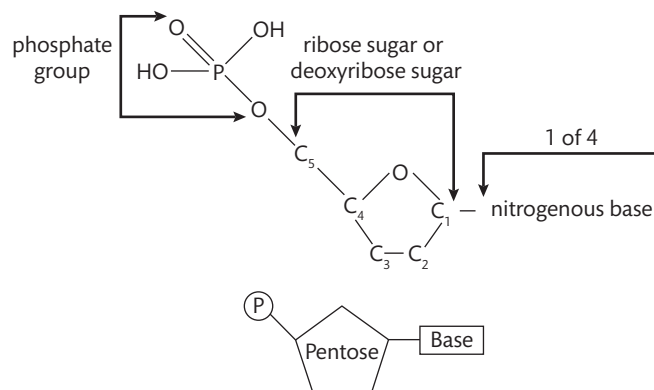
For many years most scientists all over the world believed it was protein, not DNA, that contained our genetic information. Research conducted in the first few decades of the 20th century demonstrated that DNA contains our genetic blueprint.



## Nucleotides are the building blocks of nucleic acids

As you learned earlier in this chapter, nucleic acids are one of the major carbon-based groups. There are three major examples of nucleic acids in nature. They are adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). ATP functions as an energy storage compound. Other nucleic acids function as coenzymes. In this section we will focus on DNA and RNA. DNA and RNA are involved with the genetic aspects of the cell.

Both DNA and RNA are polymers of nucleotides. Individual nucleotides are referred to as monomers and always consist of three major parts: one phosphate group, one 5-carbon monosaccharide, and a single nitrogenous base. Chemical bonds occur at specific locations in order to produce a functional unit. Look at Figure 2.29.



**Figure 2.29** The first diagram represents the structure of a nucleotide showing bond locations. The second diagram represents the structure of a general nucleotide using the symbols suggested by the IB.

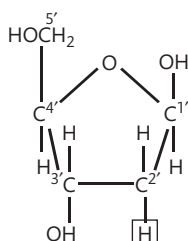
It is important to note that in the diagram circles are used to represent phosphates, pentagons are used to represent 5-carbon sugars (also called pentoses), and rectangles are used to represent nitrogenous bases. All IB drawings involving nucleotides should use these symbols.

All the bonds within the nucleotide involve the sharing of electrons, and are therefore referred to as covalent bonds. The phosphate group is the same in DNA and RNA. However, there are five possible nitrogenous bases, which are shown in Table 2.9.

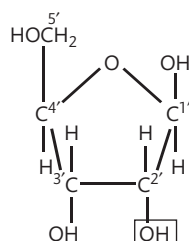
**Table 2.9 The five nitrogenous bases**

RNA nitrogenous bases	DNA nitrogenous bases
Adenine (A)	Adenine (A)
Uracil (U)	Thymine (T)
Cytosine (C)	Cytosine (C)
Guanine (G)	Guanine (G)

**Figure 2.30** Nucleotide sugars.



Deoxyribose



Ribose

The base uracil only occurs in RNA, not DNA, and the base thymine only occurs in DNA, not RNA. When drawing nucleotides, it is common practice to put the capitalized first letter of the base inside the rectangle.

The sugar differs in the nucleotides of DNA and RNA. DNA nucleotides contain the pentose known as deoxyribose and RNA nucleotides contain ribose. In Figure 2.30, you can see that they are very similar molecules.

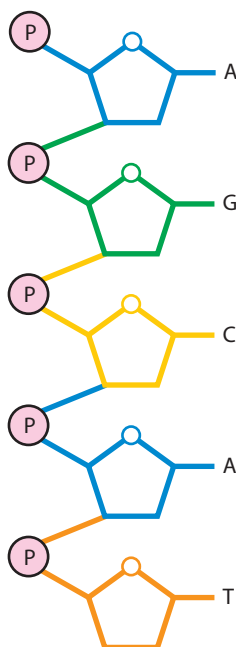
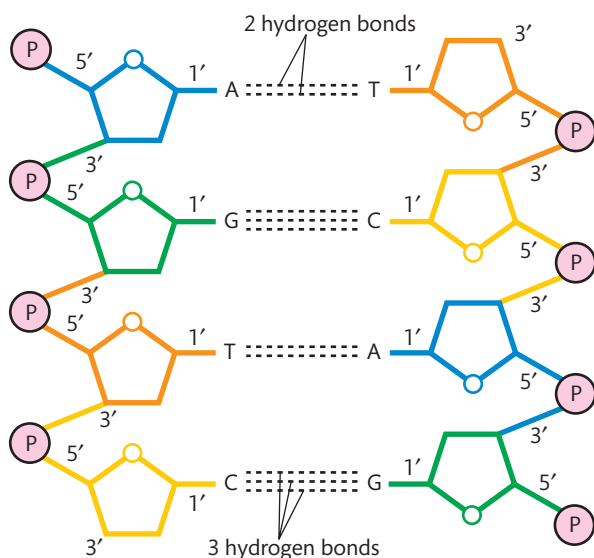
## Monomers into polymers

Monomers (single nucleotides) in both DNA and RNA may bond together to produce long chains or polymers. An example of such a chain is shown in Figure 2.31.

In Figure 2.31 each adjoining nucleotide has been drawn in a different colour to emphasize the nucleotide structure. Notice that the chain has an alternating pentose–phosphate backbone, with the nitrogenous bases extending outward. The importance of the order of these nitrogenous bases will be discussed later in conjunction with the genetic code. The nucleotides attach to one another to form a chain as a result of condensation reactions forming connecting covalent bonds.

## Single strand or double strand

RNA is composed of a single chain or strand of nucleotides, while DNA consists of two separate chains or strands of nucleotides connected to one another by weak hydrogen bonds. The strands of both DNA and RNA may involve very large numbers of nucleotides. For the two strands of DNA, imagine a double-stranded DNA molecule as a ladder (see Figure 2.32). The two sides of the ladder are made up of the phosphate and deoxyribose sugars. The rungs of the ladder (what you step on) are made up of the nitrogenous bases. Because the ladder has two sides, there are two bases making up each rung. The two bases making up one rung are said to be complementary to each other. The complementary base pairs are adenine (A)–thymine (T) and cytosine (C)–guanine (G).



## CHALLENGE YOURSELF

- 8 Use the symbols mentioned on page 86 to represent all the possible nucleotides of DNA. Once you have done that for DNA, do the same for RNA.

**Figure 2.31** Five nucleotides bonded to form a very small section of a strand of DNA or RNA.

## CHALLENGE YOURSELF

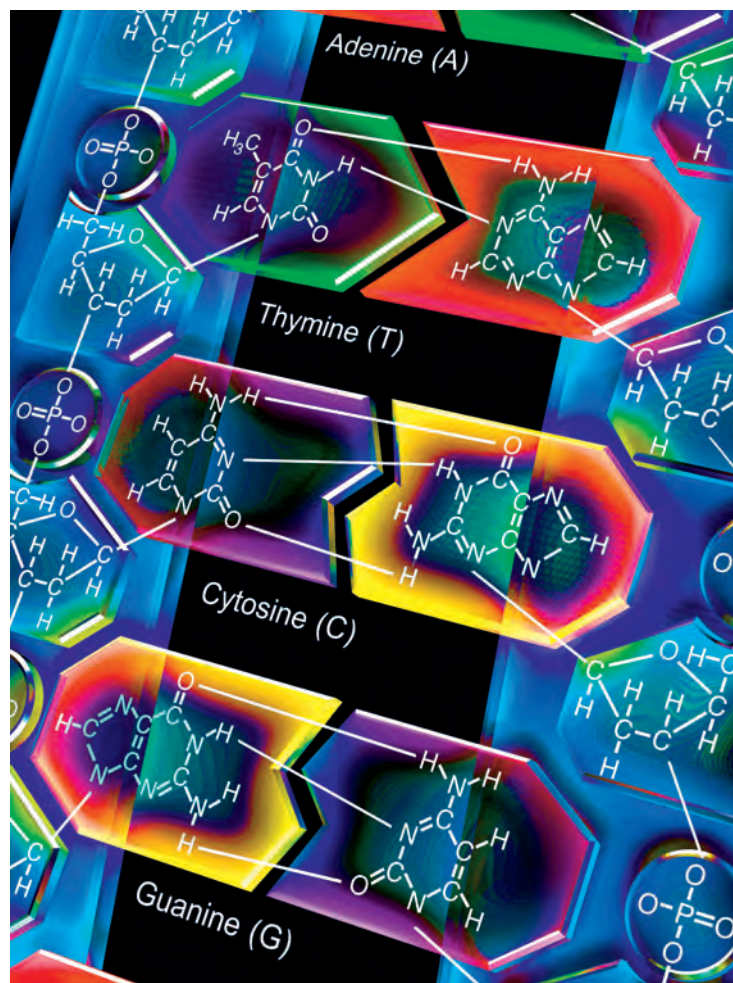
- 9 Examine the first diagram in Figure 2.29 representing the general structure of a nucleotide. Notice that the carbons of the pentose are numbered. These numbers are always placed in this way for both ribose and deoxyribose. Now look at Figure 2.31, in which five nucleotides are connected together. Answer the following.
- In the polymer, which numbered carbons are always attached to the phosphate group?
  - In a monomer, what number carbon is always attached to the phosphate group?
  - Which carbon is always attached to the nitrogenous base?

**Figure 2.32** A small section of a double-stranded DNA molecule showing hydrogen bonds between complementary nitrogenous bases. The two single strands that make up the double-stranded molecule run in opposite directions to each other. The term that describes this is 'antiparallel'. Thus we say that the two strands of the double helix are antiparallel and complementary to each other.

Even though the first accurate model of DNA was produced by James Watson (American) and Francis Crick (British) in 1953, many other scientists from around the world contributed pieces of information that were instrumental in developing the final model. Erwin Chargaff (Austrian) had determined that the numbers of adenine and thymine bases were equal, as were the numbers of cytosine and guanine bases. Rosalind Franklin (British) and Maurice Wilkins (born in New Zealand) had calculated the distance between the various molecules in DNA by X-ray crystallography.



We can now use all of this information to construct a simple, yet accurate, drawing of DNA.



**Figure 2.33** This artwork shows complementary base pairs and hydrogen bonding in DNA. Note that thymine and cytosine are much smaller molecular structures than adenine and guanine.

Even though some information was exchanged, the development of the first accurate model of DNA was highly competitive. Several groups in different parts of the world were trying to make sense of shared knowledge to produce an appropriate model. Some scientists did not share their research or findings. How is this 'anti-scientific'? Discuss what can be done to increase the sharing of personal knowledge in scientific research.

#### TOK



#### NATURE OF SCIENCE

Francis Crick and James Watson used models to arrive at the structure of DNA. They used data from many different sources to construct this model successfully. They did not have the ability to observe the molecule directly, which made the model necessary. The model they produced was an actual physical model, using wires and symbols representing atoms. Today, many models are produced using computer-based mathematical models. Regardless of how a model is produced, it is always subject to modification as more experiments are conducted and more data are collected.

## CHALLENGE YOURSELF

**10** In order to better understand the basic structures of RNA and DNA, it is useful to compare and contrast their characteristics. They are actually quite similar. When comparing two compounds, using a t-chart or a table is recommended. t-charts may take many forms, but all allow a direct comparison between related items or materials. In this case, complete the table below, which allows a comparison of the two compounds.

Feature	RNA	DNA
Number of strands		
Bases present		
Pentose present		
Name of monomers		

Table 2.10

### Exercises

- 15** Why do researchers often give DNA information as the sequence of nitrogenous bases without indicating the presence of the phosphate group and sugar component of each nucleotide?
- 16** Starting with a blank piece of paper, practise drawing a ladder diagram of DNA in which the nitrogenous base sequence of one strand is C, T, G, G, A, T. Be sure to include a representation of the phosphate groups and deoxyribose sugar in each nucleotide.

## 2.7

## DNA replication, transcription, and translation

### Understandings:

- The replication of DNA is semi-conservative and depends on complementary base pairing.
- Helicase unwinds the double helix and separates the two strands by breaking hydrogen bonds.
- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template.
- Transcription is the synthesis of mRNA copied from the DNA base sequences by RNA polymerase.
- Translation is the synthesis of polypeptides on ribosomes.
- The amino acid sequence of polypeptides is determined by mRNA according to the genetic code.
- Codons of three bases on mRNA correspond to one amino acid in a polypeptide.
- Translation depends on complementary base pairing between codons on mRNA and anticodons on tRNA.

### Application and skills:

- Application: Use of *Taq* DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR).
- Application: Production of human insulin in bacteria as an example of the universality of the genetic code allowing gene transfer between species.
- Skill: Use a table of the genetic code to deduce which codon(s) corresponds to which amino acid.
- Skill: Analysis of Meselson and Stahl's results to obtain support for the theory of semi-conservative replication of DNA.
- Skill: Use a table of mRNA codons and their corresponding amino acids to deduce the sequence of amino acids coded by a short mRNA strand of known base sequence.
- Skill: Deducing the DNA base sequence for the mRNA strand.

#### Guidance

- The different types of DNA polymerase do not need to be distinguished.



To learn more about DNA structure, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.6.



### NATURE OF SCIENCE

Obtaining evidence for scientific theories: Meselson and Stahl obtained evidence for the semi-conservative replication of DNA.



Helicase can catalyse the unzipping of DNA at a rate measured in hundreds of base pairs per second.



Helicase (currently at about the half-way point in this image of a DNA double helix being unzipped) would have started on the left and be moving towards the right.



## DNA replication involves 'unzipping'

Cells must prepare for a cell division by doubling the DNA content of the cell in a process called DNA replication. This process doubles the quantity of DNA and also ensures that there is an exact copy of each DNA molecule. In the nucleus of cells are two types of molecules that are particularly important for the process of DNA replication; they are:

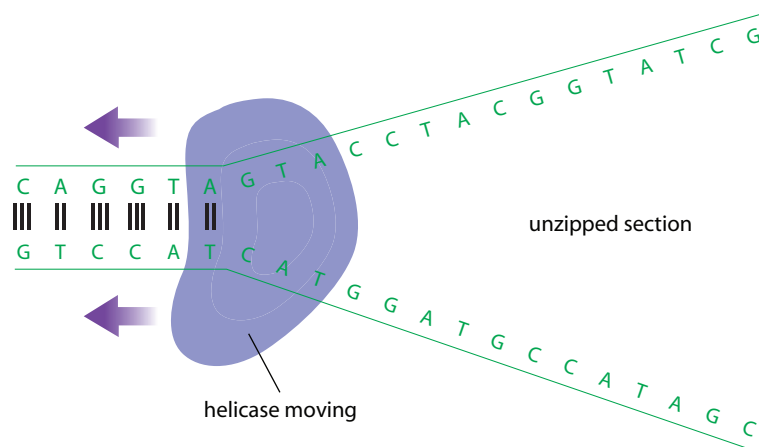
- enzymes needed for replication, which include helicase and a group of enzymes collectively called DNA polymerase
- free nucleotides, which are nucleotides that are not yet bonded and are found floating freely in the nucleoplasm, some contain adenine, some thymine, some cytosine, and some guanine.

One of the early events of DNA replication is the separation of the double helix into two single strands. You should remember that the double helix is held together by the hydrogen bonds between complementary base pairs (adenine and thymine, cytosine and guanine). The enzyme that initiates this separation into two single strands is called

helicase. Helicase begins at a point in or at the end of a DNA molecule, and moves one complementary base pair at a time, breaking the hydrogen bonds so the double-stranded DNA molecule becomes two separate strands.

The unpaired nucleotides on each of these single strands can now be used as a template to help create two double-stranded DNA molecules identical to the original. Some people use the analogy of a zipper for this process. When you pull on a zipper, helicase is like the slide mechanism. The separation of the two sides of the DNA molecule is like the two opened sides of a zipper. See Figure 2.34.

**Figure 2.34** The first step of DNA replication is helicase unzipping the double-stranded DNA molecule, forming a section with two single strands.



## Formation of two complementary strands

As shown in Figure 2.34, once DNA has become unzipped, the nitrogenous bases on each of the single strands are unpaired. In the environment of the nucleoplasm, there

are free-floating nucleotides. These nucleotides are available to form complementary pairs with the single-stranded nucleotides of the unzipped molecule. This does not happen in a random fashion. A free nucleotide locates on one opened strand at one end, and then a second nucleotide can join the first. This requires these two nucleotides to become covalently bonded together, because they are the beginning of a new strand. The formation of a covalent bond between two adjoining nucleotides is catalysed by one of the DNA polymerase enzymes that are important in this process.

A third nucleotide then joins the first two, and the process continues in a repetitive way for many nucleotides. The other unzipped strand also acts as a template for the formation of another new strand. This strand forms in a similar fashion, but in the opposite direction to the first strand. In Figure 2.35, notice that one strand is replicating in the same direction as helicase is moving and the other strand is replicating in the opposite direction.

The significance of DNA replication is that it ensures that two identical copies of DNA are produced from one original. The diagram illustrates a very small section of DNA replicating.

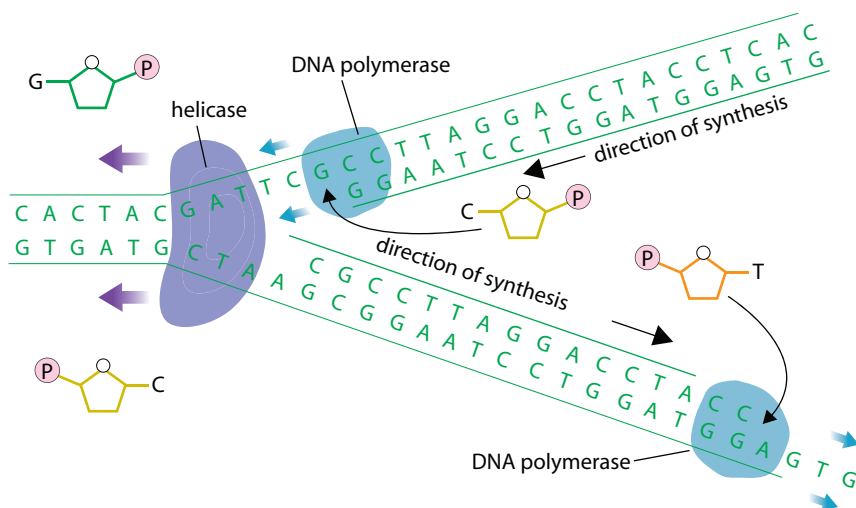
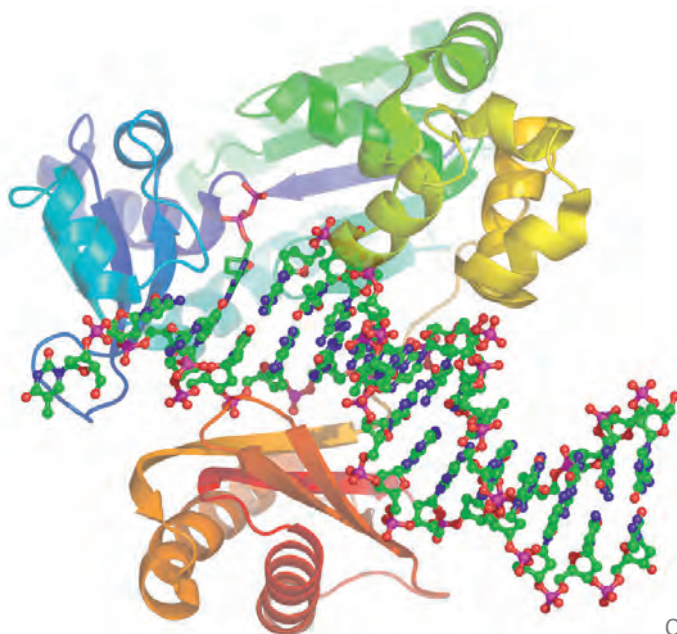


Figure 2.35 DNA replication

Figure 2.36 A small section of DNA (shown in the centre of this artwork) is seen in a DNA polymerase enzyme.

Notice that in the area where replication has already taken place, the two strands are absolutely identical to each other. This is because the original double-stranded molecule had complementary pairs of nucleotides and it was the complementary nucleotides that used the unzipped single-stranded areas as templates.

This also means that no DNA molecule is ever completely new. After replication, every DNA molecule consists of a strand that is 'old' paired with a strand that is 'new'. DNA replication is described as a semi-conservative process because half of a pre-existing DNA molecule is always conserved (saved).

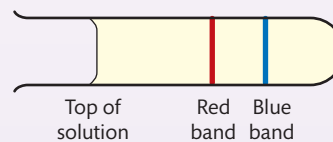


## CHALLENGE YOURSELF

**11** The experimental work that determined that DNA replication was semi-conservative is often called 'the most beautiful experiment in biology'. This experiment was carried out by Matthew Meselson and Frank Stahl, with their results published in 1958. An overview of the experiment and the data obtained follows.

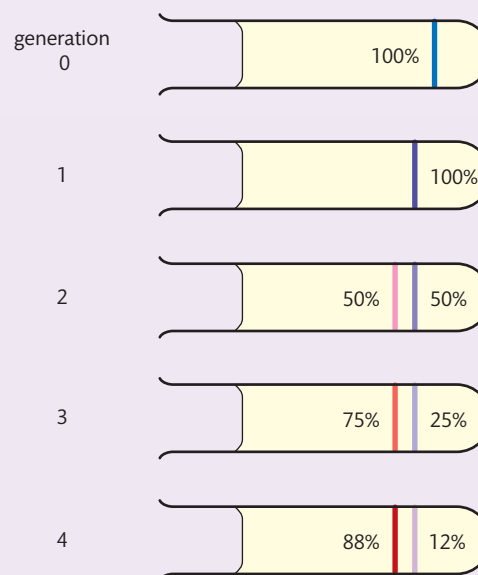
- Two separate cultures of *Escherichia coli* bacteria were grown with the presence of either a 'heavy' isotope of nitrogen,  $^{15}\text{N}$ , or an ordinary 'light' isotope of nitrogen,  $^{14}\text{N}$ .
- After many generations, the DNA in each bacteria culture contained either the heavy form or the light form of nitrogen. The nitrogen was part of the nucleotides' nitrogenous bases.
- Bacteria of each culture were treated to release their DNA into a solution.
- The solution with DNA from both cultures was then centrifuged at high speed.
- The result was two bands of DNA, the band that was lower in solution contained the  $^{15}\text{N}$ , the band that was higher contained the  $^{14}\text{N}$ .

**Figure 2.37** Meselson and Stahl's experiment.



- In Figure 2.37, you can see the two bands. The lower band had the heavier nitrogen,  $^{15}\text{N}$ .
- This first tube represented a 'standard' to which future results could be compared.
- A new culture of *E. coli* was grown in the  $^{15}\text{N}$  medium for many generations, to ensure all the DNA present was  $^{15}\text{N}$ . A DNA sample was obtained and centrifuged. This became generation 0.
- At the same time as generation 0 was obtained, some of the bacteria were placed in a  $^{14}\text{N}$  culture medium and allowed to grow for 20 minutes, which is the generation time for *E. coli* grown in optimal conditions.
- A sample was then taken, processed, and centrifuged to produce generation 1. This same process was continued so that four generations were obtained, each being processed and centrifuged.
- Figure 2.38 represents the results obtained.

**Figure 2.38** Meselson and Stahl's results.



Meselson and Stahl's experiment was performed half a century ago, but it employed techniques widely used in today's biological research. Meselson and Stahl made predictions based on a number of possible models. They then performed specially designed experiments to gather data to support one of these models.

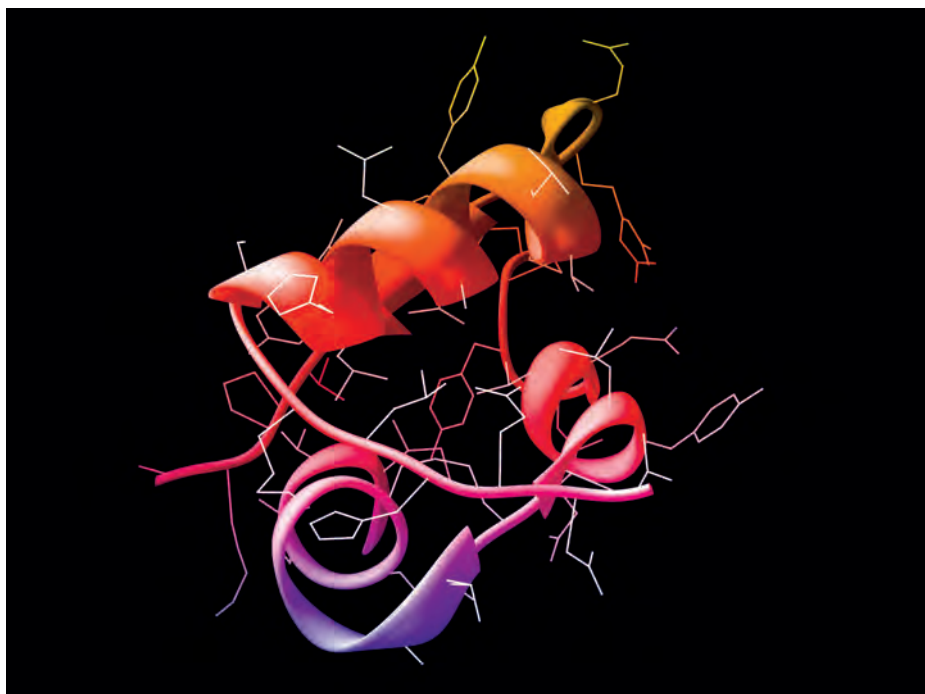
Answer these questions from the results obtained.

- (a)** In semi-conservative replication, the new molecule of DNA has one strand from the original molecule and one new strand produced from nucleotides in the surrounding environment. How does generation 1 support this model?
- (b)** Why does generation 2 support the semi-conservative model?

The results of Meselson and Stahl's experiment are summarized in Figure 2.38. Notice the colours of the original strand of DNA and how one 'parent strand' becomes one of each of the new strands produced by replication.

## Protein synthesis

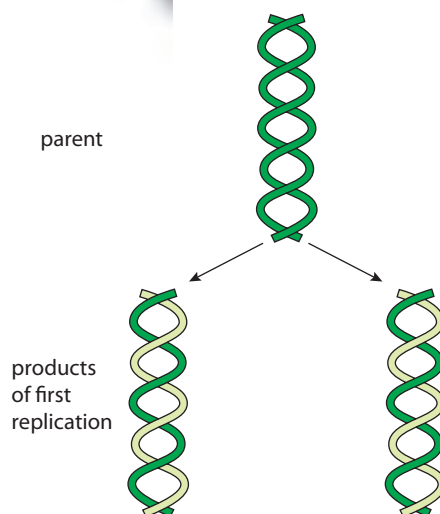
The control that DNA has over a cell is determined by a process called protein synthesis. In simple terms, DNA controls the proteins produced in a cell. Some of the proteins produced are enzymes. The production (or lack of production) of a particular enzyme can have a dramatic effect on the overall biochemistry of the cell. Thus DNA indirectly controls the biochemistry of carbohydrates, lipids, and nucleic acids with the production of enzymes.



Protein synthesis involves two major sets of reactions, transcription and translation. Both either produce or require a type of nucleic acid called RNA, which was discussed in Section 2.6.

## Transcription produces RNA molecules

The sections of DNA that code for polypeptides are called genes. Any one gene is a specific sequence of nitrogenous bases found in a specific location in a DNA



**Figure 2.39** This figure demonstrates the general process of semi-conservative replication of DNA.

This computer graphic shows an insulin molecule. Insulin is a protein hormone and is produced by protein synthesis.



molecule. Molecules of DNA are found within the confines of the nucleus, yet proteins are synthesized outside the nucleus in the cytoplasm. This means that there has to be an intermediary molecule that carries the message of the DNA (the code) to the cytoplasm where the enzymes, ribosome, and amino acids are found. This intermediary molecule is called messenger RNA (mRNA).

The nucleoplasm (fluid in the nucleus) contains free nucleotides, as mentioned earlier. In addition to the free nucleotides used for DNA replication, the nucleoplasm also contains free RNA nucleotides. Each of these is different from the DNA counterpart, because RNA nucleotides contain the sugar ribose not deoxyribose. Another major difference is that no RNA nucleotides contain thymine; instead there is a nitrogenous base unique to RNA, called uracil.

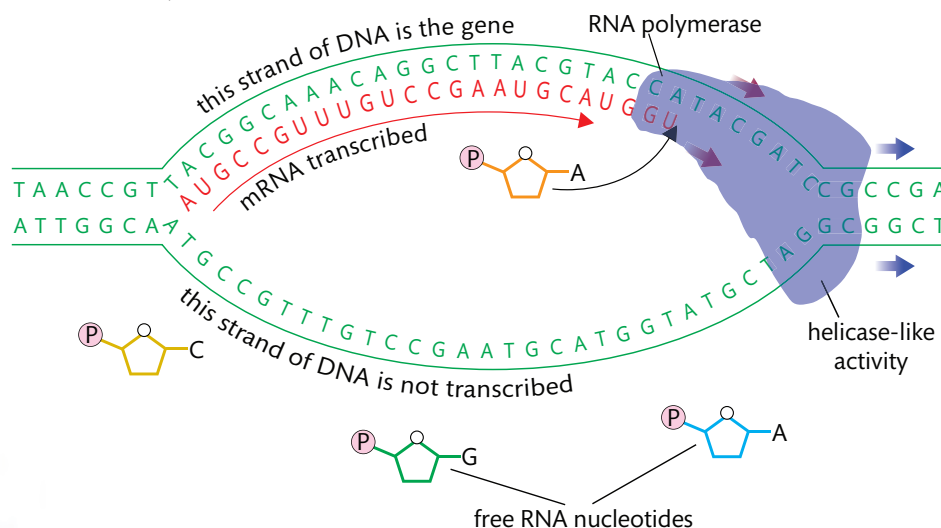
### The transcription process

The process of transcription begins when an area of DNA of one gene becomes unzipped (see Figure 2.40). This is very similar to the unzipping process involved in DNA replication, but in this case only the area of the DNA where the particular gene is found is unzipped. The two complementary strands of DNA are now single-stranded in the area of the gene. Recall that RNA (which includes mRNA) is a single-stranded molecule. This means that only one of the two strands of DNA will be used as a template to create the mRNA molecule. An enzyme called RNA polymerase is used as the catalyst for this process.

As RNA polymerase moves along the strand of DNA acting as the template, RNA nucleotides float into place by complementary base pairing. The complementary base pairs are the same as in double-stranded DNA, with the exception that adenine on the DNA is now paired with uracil on the newly forming mRNA molecule. Consider the following facts concerning transcription:

- only one of the two strands of DNA is 'copied,' the other strand is not used
- mRNA is always single-stranded and shorter than the DNA that it is copied from, as it is a complementary copy of only one gene
- the presence of thymine in a molecule identifies it as DNA (the presence of deoxyribose is another clue)
- the presence of uracil in a molecule identifies it as RNA (the presence of ribose is another clue).

**Figure 2.40** Transcription (synthesis of an RNA molecule). RNA polymerase has helicase-like activity as it plays a role in opening the DNA double helix. It also catalyses the addition of free RNA nucleotides to the growing mRNA strand.



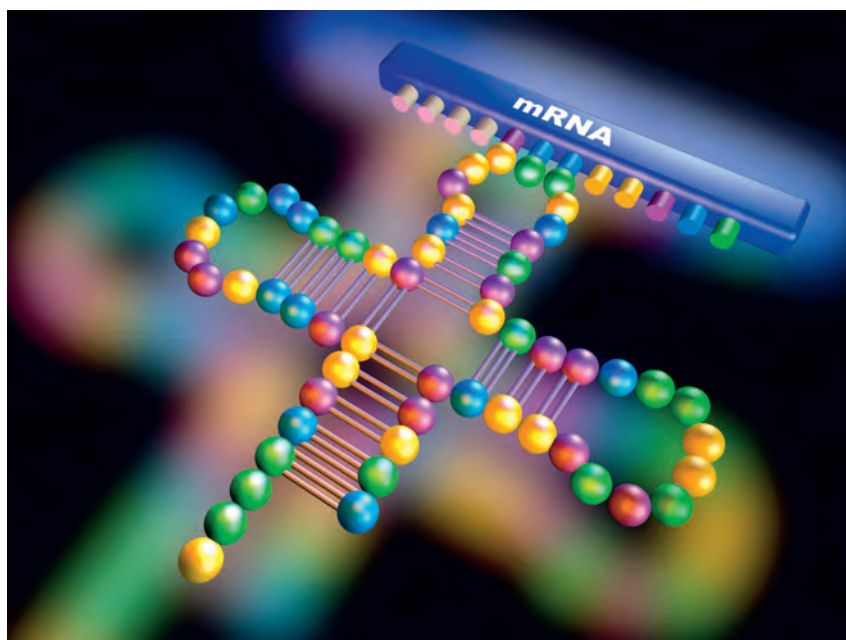
## The genetic code is written in triplets



The mRNA molecule produced by transcription represents a complementary copy of one gene of DNA. The sequence of mRNA nucleotides is the transcribed version of the original DNA sequence. This sequence of nucleotides making up the length of the mRNA is typically enough information to make one polypeptide. As you will recall, polypeptides are composed of amino acids covalently bonded together in a specific sequence. The message written into the mRNA molecule is the message that determines the order of the amino acids. Researchers found experimentally that the genetic code is written in a language of three bases. In other words, a set of three bases contains enough information to code for one of the 20 amino acids. Any set of three bases that determines the identity of one amino acid is called a triplet. When a triplet is found in an mRNA molecule, it is called a codon or codon triplet. This is shown in the model below.

## Translation results in the production of a polypeptide

There are three different kinds of RNA molecule. They are all single-stranded and each is transcribed from a gene (a section of DNA).



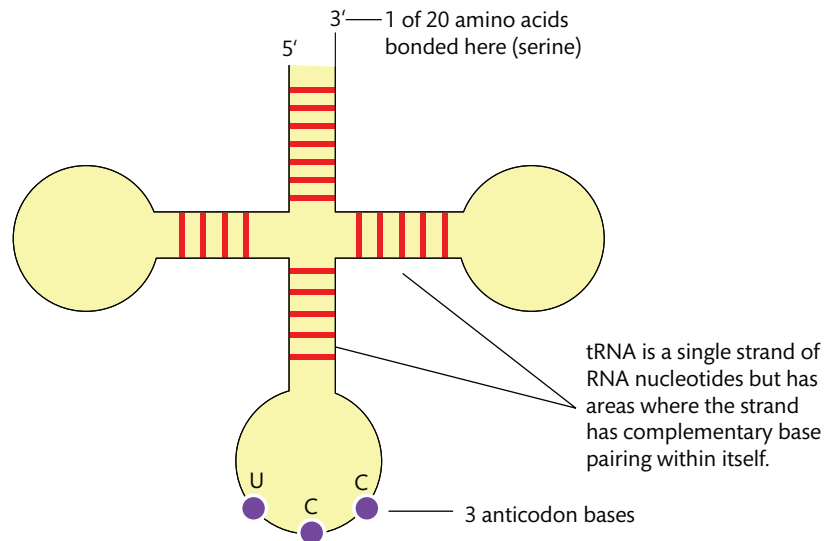
In this model, you can see mRNA (upper right) and tRNA (the clover shape). The amino acid that would be bonded to the tRNA is not shown.

Here is a quick summary of each RNA type:

- mRNA, messenger RNA, as described above, each mRNA is a complementary copy of a DNA gene and has enough genetic information to code for a single polypeptide
- rRNA, ribosomal RNA, each ribosome is composed of rRNA and ribosomal protein
- tRNA, transfer RNA, each type of tRNA transfers one of the 20 amino acids to the ribosome for polypeptide formation.

Figure 2.41 shows a typical tRNA molecule. Notice that the three bases in the middle loop are called the anticodon bases, and they determine which of the 20 amino acids is attached to the tRNA.

**Figure 2.41** Structure of a tRNA 3' molecule.



Once an mRNA molecule has been transcribed, the mRNA detaches from the single-strand DNA template and floats free in the nucleoplasm. At some point, the mRNA will float through one of the many holes in the nuclear membrane (nuclear pores) and will then be in the cytoplasm.

### The translation process

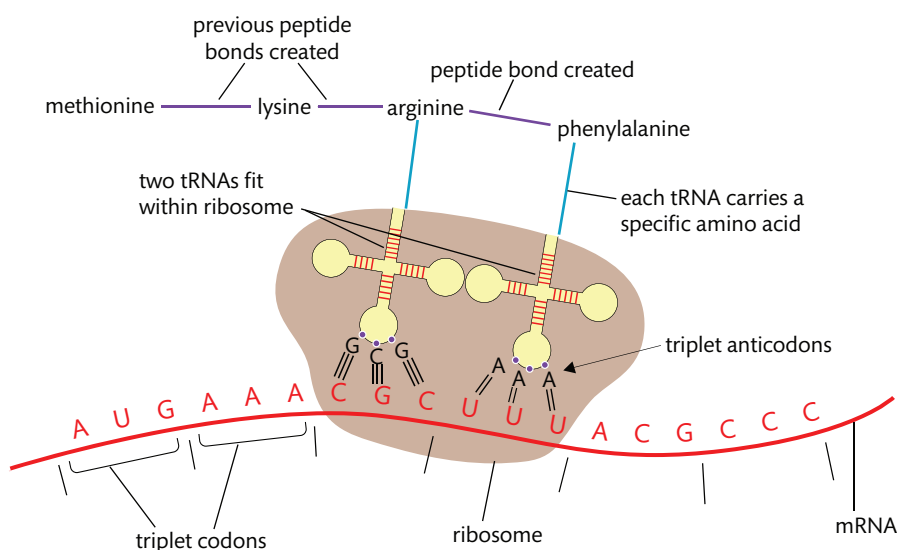
The mRNA will locate a ribosome and align with it, so that the first two codon triplets are within the boundaries of the ribosome.

A specific tRNA molecule now floats in: its tRNA anticodon must be complementary to the first codon triplet of the mRNA molecule. Thus the first amino acid is brought into the translation process. It is not just any amino acid: its identity was originally determined by the strand of DNA that transcribed the mRNA being translated. While the first tRNA 'sits' in the ribosome holding the first amino acid, a second tRNA floats in and brings a second (again specific) amino acid. The second tRNA matches its three anticodon bases with the second codon triplet of the mRNA. As you can see in Figure 2.42, two specific amino acids are now being held side by side. An enzyme then catalyses a condensation reaction between the two amino acids, and the resulting covalent bond between them is called a peptide bond.

The next step in the translation process involves breaking the bond between the first tRNA molecule and the amino acid that it transferred in. This bond is no longer needed, as the second tRNA is currently bonded to its own amino acid, and that amino acid is covalently bonded to the first amino acid. The first tRNA floats away into the cytoplasm and invariably reloads with another amino acid of the same type. The ribosome that has only one tRNA in it now moves one codon triplet down the mRNA molecule. This, in effect, puts the second tRNA in the ribosome position that the first originally occupied, and creates room for a third tRNA to float in, bringing with it a third specific amino acid. The process now becomes repetitive: as another

The process of producing proteins utilizes a DNA code that is universal in all organisms. Because of this, researchers have successfully inserted the human gene that codes for the production of human insulin into bacteria. The result of this is bacteria that produce human insulin that can be used to treat humans with diabetes.

peptide bond forms, the ribosome moves on by another triplet, and so on. The process continues until the ribosome gets to the last codon triplet. The final codon triplet will be a triplet that does not act as a code for an amino acid, instead it signals 'stop' to the process of translation. The entire polypeptide breaks away from the final tRNA molecule, and becomes a free-floating polypeptide in the cytoplasm of the cell.



**TOK**

Who should decide how fast and how far humans should go with our study of DNA and the technology that is rapidly emerging?

**Figure 2.42** Events of translation (synthesis of a polypeptide).



To learn more about DNA replication and transcription and to find a codon chart, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.7.

## Polymerase chain reaction and *Taq* DNA polymerase

Polymerase chain reaction, also known as PCR, was developed in the 1970s. It is a means by which DNA replication can be carried out artificially in a laboratory setting. However, it can only replicate rather short segments of DNA. By replicating DNA segments, scientists can produce huge numbers of these segments to study and analyse. It is often used in forensic situations when only a limited amount of the original DNA has been recovered at a crime scene.

An enzyme is used in PCR that is stable at relatively high temperatures. This enzyme was discovered in 1985 from a bacterium called *Thermus aquaticus* (*Taq*). This bacterium occurs naturally in hot springs, and its enzymes are not denatured at high temperatures, including the specific DNA polymerase that it possesses. This DNA polymerase has been named *Taq* polymerase and its use has greatly increased the number of discoveries in the field of gene technology.

### Exercises

- 17 What type of bonds does helicase act upon?
- 18 What is the difference between a codon and a triplet?
- 19 What are the two major sets of reactions in protein synthesis?
- 20 What are the three major parts of all nucleotides?

## CHALLENGE YOURSELF

- 12** Imagine that an mRNA leaves the nucleus of a eukaryotic cell with the following base sequence:  
AUGCCCCGCACGUUCC  
AAGCCCCGGG.

Find an mRNA codon chart and answer the following.

- (a) Determine in sequence the amino acids that are coded for by the above mRNA molecule.
- (b) Determine the DNA code sequence that gave rise to the above mRNA codons.
- (c) What would the amino acid sequence be if the first cytosine of the mRNA molecule was replaced with a uracil? (This would be the result of a change occurring in the DNA molecule that transcribed this mRNA.)



## NATURE OF SCIENCE

Assessing the ethics of scientific research: the use of invertebrates in respirometer experiments has ethical implications.



## 2.8 Cell respiration

## Understandings:

- Cell respiration is the controlled release of energy from organic compounds to produce ATP.
- ATP from cell respiration is immediately available as a source of energy in the cell.
- Anaerobic cell respiration gives a small yield of ATP from glucose.
- Aerobic cell respiration requires oxygen and gives a large yield of ATP from glucose.

## Applications and skills:

- Application: Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.
- Application: Lactate production in humans when anaerobic respiration is used to maximize the power of muscle contractions.
- Skill: Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer.

## Guidance

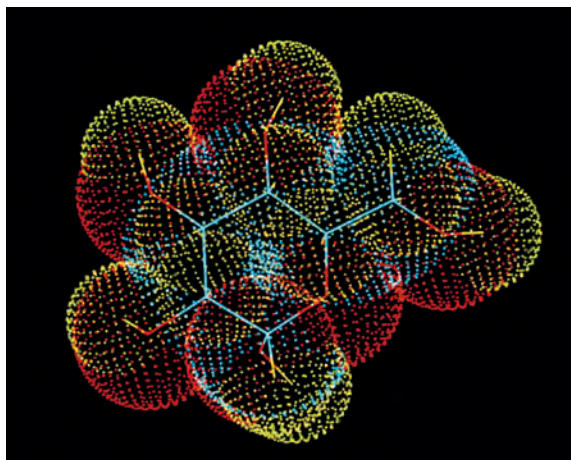
- Details of the metabolic pathways of cell respiration are not needed but the substrates and final waste products should be known.
- There are many simple respirometers that could be used. Students are expected to know that an alkali is used to absorb carbon dioxide, so reductions in volume are due to oxygen use. Temperature should be kept constant to avoid volume changes due to temperature fluctuations.

## Cell respiration is used by all cells to produce ATP

Organic molecules contain energy in their molecular structures. Each covalent bond in a glucose, amino acid, or fatty acid represents stored chemical energy. When we burn wood in a fire, we are releasing the stored chemical energy in the form of heat and light. Burning is the release of chemical energy called rapid oxidation.

Cells break down (or metabolize) their organic nutrients by slow oxidation. A molecule, such as glucose, is acted on by a series of enzymes. The function of these enzymes is to catalyse a sequential series of reactions in which the covalent bonds are broken (oxidized) one at a time. Each time a covalent bond is broken, a small amount of energy is released. The ultimate goal of releasing energy in a controlled way is to trap the released energy in the form of ATP molecules. If a cell does not have glucose available, other organic molecules may be substituted, such as fatty acids or amino acids.

This is a computer graphic of glucose. The backbone of the molecule is shown in stick form. The spheres represent the relative sizes of the individual atoms ( $C_6H_{12}O_6$ ).

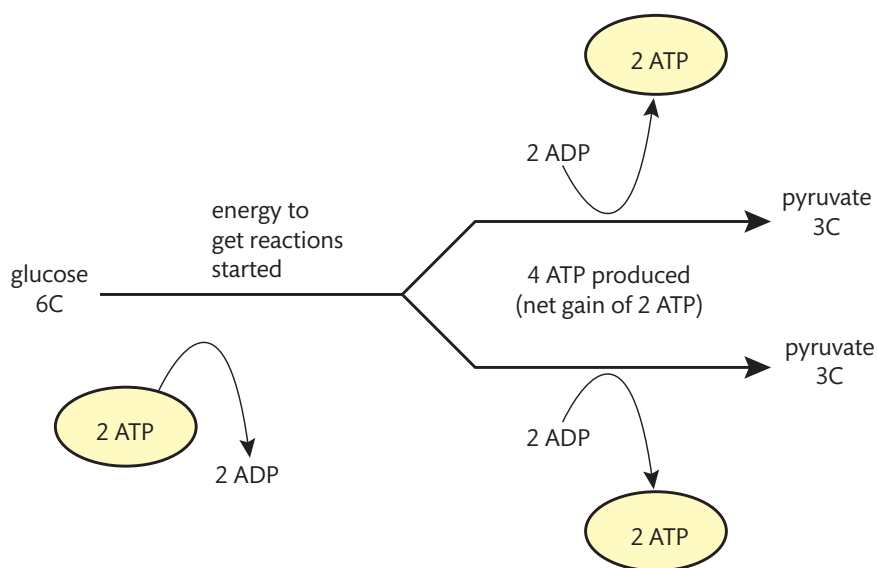


## Glycolysis is the first step in the cell respiration process

Assuming that glucose is the organic nutrient being metabolized, all cells begin the process of cell respiration in the same way. Glucose enters a cell through the plasma membrane and floats in the cytoplasm. An enzyme modifies the glucose slightly, then a second enzyme modifies this molecule even more. This is followed by an entire series of reactions that ultimately cleaves the 6-carbon glucose into two 3-carbon molecules. Each of these 3-carbon molecules is called pyruvate. Some, but certainly not all, of the covalent bonds in the glucose are broken during this series of reactions. Some of the energy that is released from the breaking of these bonds is used to form a small number of ATP molecules. Notice in Figure 2.43 that two ATP molecules are needed to begin the process of glycolysis and a total of four ATP molecules are formed. This is referred to as a net gain of two ATP (a gain of four ATP minus the two ATP needed at the start).

## Some cells use anaerobic respiration for ATP production

The term 'cell respiration' refers to a variety of biochemical pathways that can be used to metabolize glucose. All of the pathways start with glycolysis. In other words, glycolysis is the metabolic pathway that is common to all organisms on Earth. Some organisms derive their ATP completely without the use of oxygen and are referred to as anaerobic. The breakdown of organic molecules for ATP production in an anaerobic way is also called fermentation. There are two main anaerobic pathways, which will be discussed here separately: alcoholic fermentation and lactic acid fermentation.



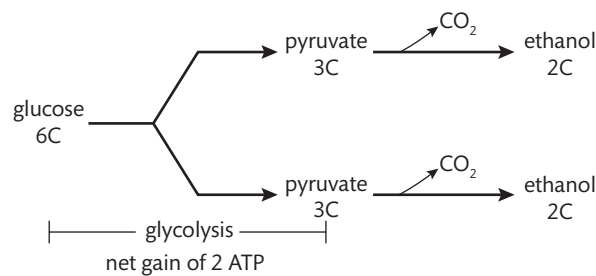
**Figure 2.43** A simplified version of the events of glycolysis.

Most types of yeast are facultatively anaerobic, which means they only carry out alcoholic fermentation when oxygen is not available. If oxygen is present they actually carry out a different type of respiration, in which ethanol and carbon dioxide are not produced. Yeast cells are eukaryotic and do possess mitochondria.



### Alcoholic fermentation

Yeast is a common single-cell fungus that uses alcoholic fermentation for ATP generation when oxygen is not present (see Figure 2.44). You will recall that all organisms use glycolysis to begin the cell respiration sequence. Thus yeast cells take in glucose from their environment and generate a net gain of two ATP by glycolysis. The organic products of glycolysis are always two pyruvate molecules. Yeast then converts both of the 3-carbon pyruvate molecules to molecules of ethanol. Ethanol is a 2-carbon molecule, so a carbon atom is 'lost' in this conversion. The 'lost' carbon atom is given off in a carbon dioxide molecule. Both the ethanol and carbon dioxide that are produced are waste products from the yeast and are simply released into the environment. Bakers' yeast is added to bread products for baking because the generation of carbon dioxide helps the dough to rise. It is also common to use yeast in the production of ethanol as alcohol to be drunk.



**Figure 2.44** A simplified version of the events of alcoholic fermentation.

All alcohol that is sold to be drunk is ethanol. Beer, wine, and spirits contain different proportions of ethanol, plus other ingredients for flavouring.



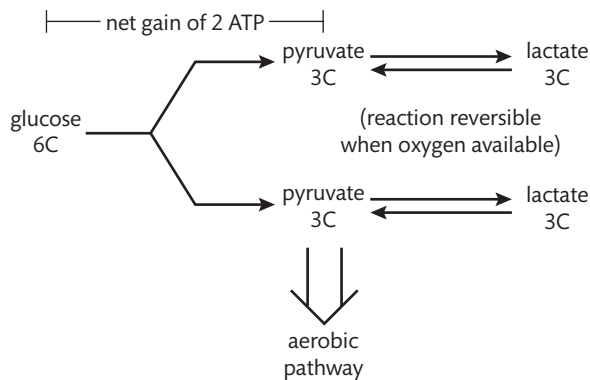
### Lactic acid fermentation

Organisms that normally use a cell respiration pathway that involves oxygen sometimes find themselves in a metabolic situation where they cannot supply enough oxygen to their cells. A good example of this is a person exercising beyond his or her normal pattern or routine. In this situation, the person's pulmonary and cardiovascular systems (lungs and heart) supply as much oxygen to the body's cells as is physically possible. If the person's exercise rate then exceeds his or her body's capacity to supply oxygen, at least some of the glucose entering into cell respiration will follow the anaerobic pathway called lactic acid fermentation. See Figure 2.45.

Once again, recall that glycolysis is used by all cells to begin the cell respiration sequence. Also remember that glycolysis:

- takes place in the cytoplasm
- results in the net gain of two ATP molecules per glucose molecule
- results in the production of two pyruvate molecules.

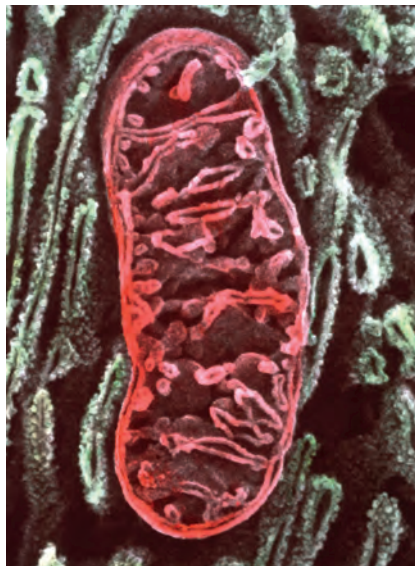
Cells that are aerobic normally take the two pyruvate molecules and metabolize them further in an aerobic series of reactions. But if cell is not getting a sufficient amount of oxygen for the aerobic pathway, i.e. is in a low-oxygen situation, excess pyruvate molecules are converted into lactic acid molecules. Like pyruvate, lactic acid molecules are 3-carbon molecules, so there is no production of carbon dioxide. What benefit does this serve? Lactic acid fermentation allows glycolysis to continue with a small gain of ATP in addition to the ATP that is generated through the aerobic pathway.



**Figure 2.45** A simplified version of the events of lactic acid fermentation.

## Aerobic cell respiration is the most efficient pathway

Cells that have mitochondria usually use an aerobic pathway for cell respiration. This pathway also begins with glycolysis, and thus has a net gain of two ATP as well as generating two pyruvate molecules. The two pyruvate molecules then enter a mitochondrion and are metabolized further.



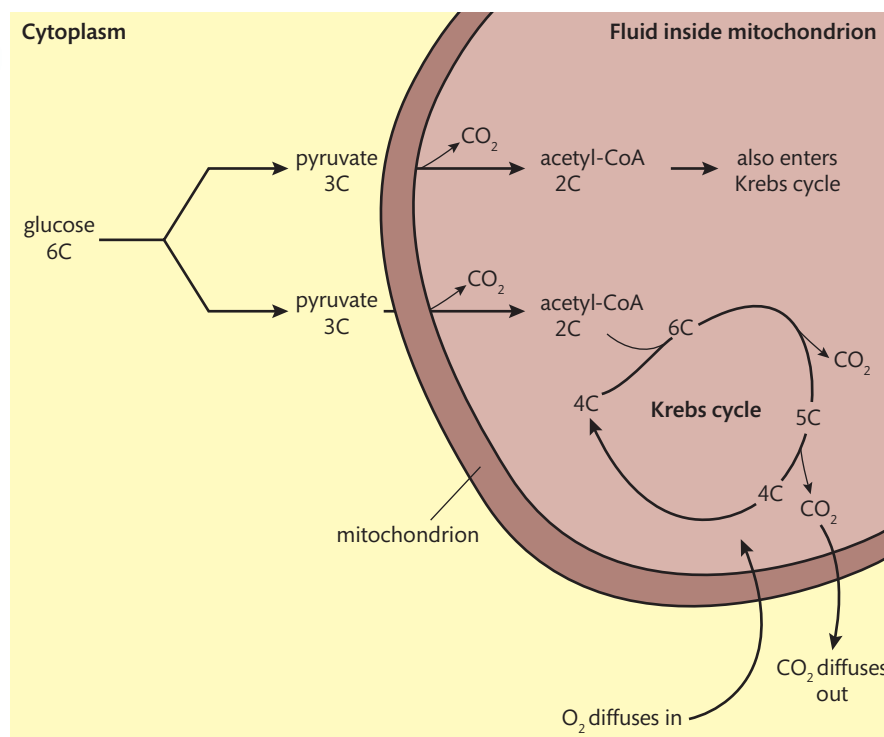
This high-resolution, false-colour SEM shows a single mitochondrion. Any cell containing mitochondria uses aerobic cell respiration as its primary cell respiration pathway.

Each pyruvate first loses a carbon dioxide molecule and becomes a molecule known as acetyl-CoA. Each acetyl-CoA molecule enters into a series of reactions called the Krebs cycle. During this series of reactions, two more carbon dioxide molecules are produced from each original pyruvate molecule that entered it. The Krebs cycle is said to be a cycle because it is a series of chemical reactions that begin and end with the same molecule. This reacquisition of the beginning molecule allows this series of chemical reactions to be repeated over and over again (see Figure 2.46).

Some ATP is generated directly during the Krebs cycle and some is generated indirectly through a later series of reactions directly involving oxygen. Aerobic cell respiration breaks down (or completely oxidizes) a glucose molecule and the end-products are carbon dioxide and water plus a much higher number of ATP molecules than anaerobic respiration yields.

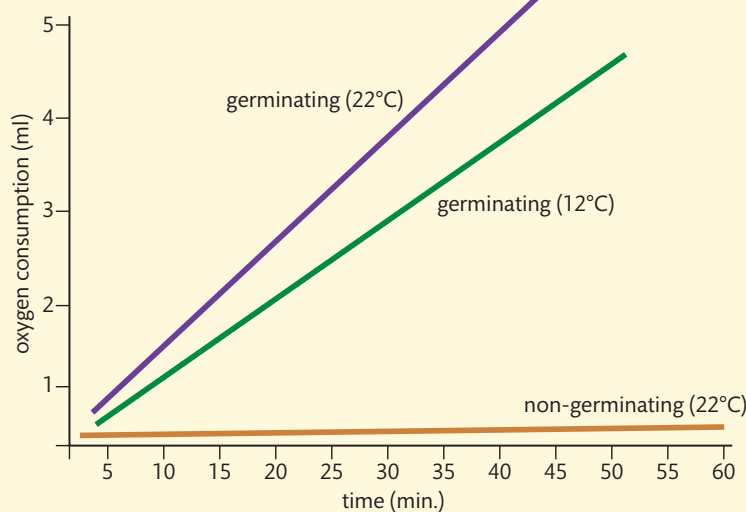


**Figure 2.46** Aerobic cell respiration. Notice that the 4C molecule of the Krebs cycle combines with the 2C molecule called acetyl-CoA. The resulting 6C molecule then goes through a series of reactions in which two carbons are lost in the form of carbon dioxide. This restores the 4C molecule that can begin the cycle all over again.



### Worked example

Respirometers are devices used to measure an organism's rate of respiration by measuring the oxygen rate of exchange. They are sealed units in which any carbon dioxide produced is absorbed by an alkali such as soda lime or potassium hydroxide. Absorbing the carbon dioxide allows an accurate measurement of oxygen exchange. These devices may work at a cellular level or at a whole-organism level. Look at the graph and answer the questions. The y-axis of the graph represents the relative amount of oxygen used.



**Figure 2.47** Oxygen consumption by germinating and non-germinating pea seeds at 12°C and 22°C.

- 1 In the germinating pea seeds, what type of respiration is occurring? What is the evidence for this answer?
- 2 Why is the oxygen consumption of non-germinating pea seeds very low?

3 Why would the germinating seeds show a greater oxygen consumption at 22°C than at 12°C?

4 Predict how the graph would look for non-germinating seeds at 12°C.

### Solutions

1 Aerobic. There is a significant amount of oxygen consumption occurring.

2 They are not carrying out respiration and have a low metabolic rate.

3 At 22°C the rate of respiration is faster than at 12°C. Therefore, there is a greater oxygen consumption at the higher temperature.

4 The line of the graph would be almost right on the non-germinating (22°C) line that exists now. A prediction that it would be just slightly lower is best.

### NATURE OF SCIENCE

It is tempting to place invertebrates in respirometers to determine oxygen consumption. However, the use of invertebrates in such experiments has ethical implications. It is essential to refer to the IB animal experimental policy before carrying out any procedures on animals. A discussion of the ethics of animal use in respirometer experiments would be wise at this point.



### Exercises

21 Which stage of cell respiration is common to all types of cell respiration?

22 Where does this stage of cell respiration occur in a cell?

23 Why does that make sense?

24 Why do we inhale oxygen and exhale carbon dioxide?



To learn more about aerobic cell respiration, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.8.

## 2.9 Photosynthesis

### Understandings:

- Photosynthesis is the production of carbon compounds in cells using light energy.
- Visible light has a range of wavelengths, with violet the shortest wavelength and red the longest.
- Chlorophyll absorbs red and blue light most effectively, and reflects green light more than other colours.
- Oxygen is produced in photosynthesis from the photolysis of water.
- Energy is needed to produce carbohydrates and other carbon compounds from carbon dioxide.
- Temperature, light intensity, and carbon dioxide concentration are possible limiting factors on the rate of photosynthesis.

### Applications and skills:

- Application: Changes to the Earth's atmosphere, oceans, and rock deposition due to photosynthesis.
- Skill: Drawing an absorption spectrum for chlorophyll, and an action spectrum for photosynthesis.
- Skill: Design of experiments to investigate the effect of limiting factors on photosynthesis.
- Skill: Separation of photosynthetic pigments by chromatograph.

#### Guidance

- Students should know that visible light has wavelengths between 400 and 700 nm, but they are not expected to recall the wavelengths of specific colours of light.
- Water free of dissolved carbon dioxide for photosynthesis experiments can be produced by boiling and cooling water.
- Paper chromatography can be used to separate photosynthetic pigments but thin layer chromatography gives better results.



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Experimental design: controlling relevant variables in photosynthesis experiments is essential.

## Photosynthesis converts light energy into chemical energy

Plants and other photosynthetic organisms produce foods that start food chains. We count on the Sun as a constant energy source for both warmth and food production for all of our planet. However, the sunlight that strikes Earth must be converted into a form of chemical energy in order to be useful to all non-photosynthetic organisms. The most common chemical energy produced from photosynthesis is the molecule glucose. If you recall, glucose is also the most common molecule that organisms use for fuel in the process of cell respiration.

## Plants use the pigment chlorophyll to absorb light energy

The vast majority of plant leaves appear green to our eyes. If you were able to zoom into leaf cells and look around, you would see that the only structures in a leaf that are actually green are the chloroplasts. Plants contain a variety of pigments in chloroplasts. The photosynthetic pigment that dominates in most plant species is the molecule chlorophyll.



### Separation of photosynthetic pigments by chromatograph

*\*Safety alerts: Fumes from the chemicals used in this procedure are dangerous. Use the chemicals in a well-ventilated area or under an exhaust or fume hood. Wear goggles and a lab apron throughout the procedure. Follow all your teacher's specific instructions.\**

As stated above, many plants contain a variety of pigments. A procedure known as paper chromatography can separate the pigments present in most modern plants. The pigment called chlorophyll *a* is the principal pigment. Chlorophyll *b*, carotenes, and xanthophylls act as accessory pigments by absorbing light at different wavelengths, and passing this energy on to chlorophyll *a* to be used in photosynthesis.

- Spinach, *Spinacia oleracea*, or kale, *Brassica oleracea*, leaves are recommended for this procedure. A chromatography solvent that consists of an organic solvent, such as a type of alcohol, acetone, or petroleum ether, will be used. Be very careful with the solvent, it is highly flammable and should be worked with under some type of a fume or exhaust hood. A strip of chromatography paper must be used for this lab as well.
- Place a line of pigment from the leaf on a strip of chromatography paper using a 'ribbed' coin. This line should be dark in colour and as thin as possible. Several repeated applications with the coin at the same place on the paper should result in a dark-coloured line.
- The paper, with a pencil mark where the pigment was placed, is then positioned inside a closed chromatography chamber filled with a shallow layer of chromatography solvent. This solvent layer should reach between the tip of the paper and the pencil line for the pigment. The paper is then placed in the closed chromatography chamber until the solvent comes to within 1–2 cm of the top of the paper.
- When the solvent has reached this position on the paper, remove the paper from the chamber, keeping all parts under the exhaust or fume hood, and immediately mark the position of the solvent line. Mark the positions of the different coloured pigments on the paper.
- Now calculate the  $R_f$  value for each of the separated pigments.

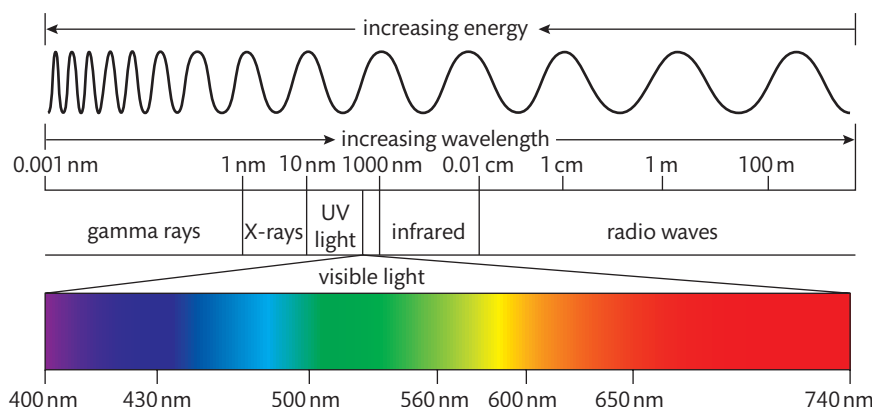
$R_f$  refers to retention factor or relative mobility factor.  $R_f$  = distance moved by pigment/distance moved by solvent. Record your results below.

Pigment colour	Distance solvent moved	Distance pigment moved	R <sub>f</sub> value
Carotene (orange)			
Xanthophylls (yellow)			
Light green (chlorophyll a)			
Green (chlorophyll b)			

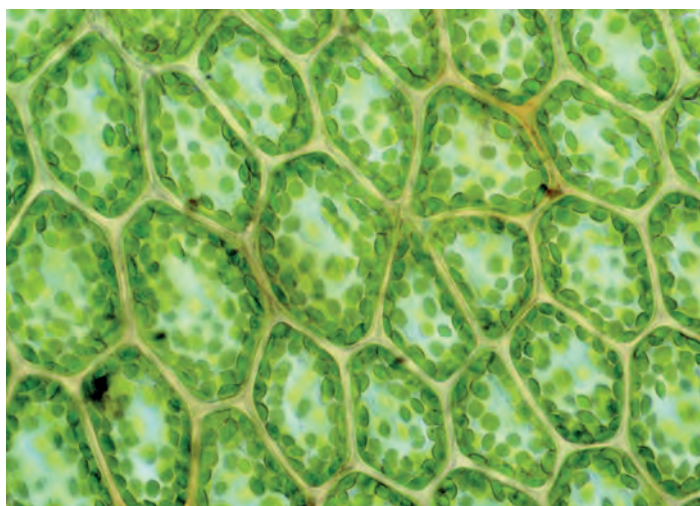
- 1 Explain why the four pigments moved at different rates through the chromatography paper.
- 2 Would any leaf from any plant have each of the pigments that are present in spinach or kale? How would this affect the chromatograph of these different leaves?
- 3 A procedure known as thin layer chromatography would give even better results than chromatography paper. Research and explain the difference between thin layer chromatography and paper chromatography.

**Table 2.11** Lab results

Plants make use of the same part of the electromagnetic spectrum that our eyes are able to see. We call this the visible portion of the spectrum. Sunlight is actually a mixture of different colours of light. You can see these colours when you let sunlight pass through a prism.



**Figure 2.48** The electromagnetic spectrum. Notice that the visible light portion of this spectrum has colours with wavelengths of between 400 nm and 740 nm.



Inside each of these plant leaf cells are many green chloroplasts. Each chloroplast is loaded with chlorophyll.



The visible light spectrum includes many colours, but, for the purpose of considering how chlorophyll absorbs light energy, we are going to consider three regions of the spectrum:

- the red end of the spectrum
- the green middle of the spectrum
- the blue end of the spectrum.

Substances can do one of only two things when they are struck by a particular wavelength (colour) of light. They can:

- absorb that wavelength (if so, energy is being absorbed and may be used)
- reflect that wavelength (if so, the energy is not being absorbed and you will see that colour).

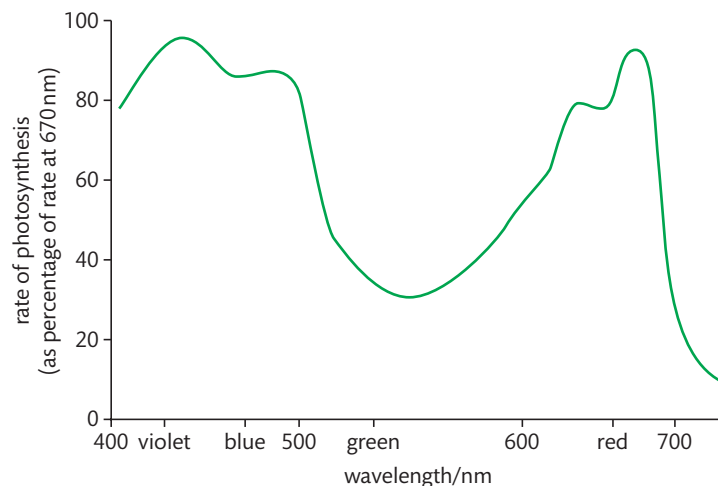
### Worked example

You are walking outside with a friend who is wearing a red and white shirt. Explain why the shirt appears to be red and white.

### Solution

Sunlight is a mixture of all of the wavelengths (colours) of visible light. When sunlight strikes the red pigments in the shirt, the blue and the green wavelengths of light are absorbed, but the red wavelengths are reflected. Thus, our eyes see red. When sunlight strikes the white areas of the shirt, all the wavelengths of light are reflected and our eyes and brain interpret the mixture as white.

Let's apply this information to how chlorophyll absorbs light for photosynthesis. Chlorophyll is a green pigment. This means that chlorophyll reflects green light and therefore must absorb the other wavelengths of the visible light spectrum. When a plant leaf is hit by sunlight, the red and blue wavelengths of light are absorbed by chlorophyll and used for photosynthesis. Almost all the energy of the green wavelengths is reflected, not absorbed.



**Figure 2.49** This action spectrum of photosynthesis indicates that most photosynthesis occurs in the blue and the red wavelength areas. Note the lower rate of photosynthesis with the green wavelength.

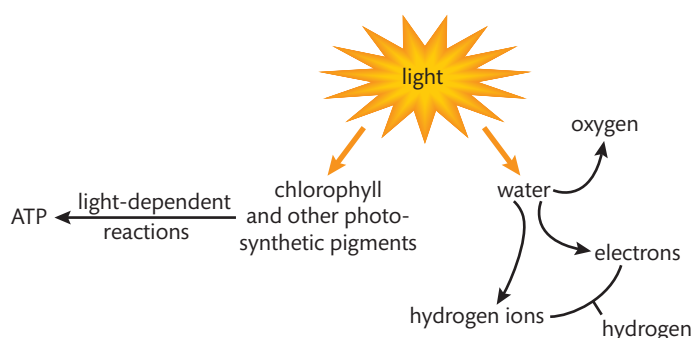
## Photosynthesis occurs in two stages

Photosynthesis produces sugar molecules as a food source for the plant. Sugars, such as glucose, are held together by covalent bonds. It requires energy to create those covalent bonds, and the source of that energy can ultimately be traced back to the Sun.

The first stage of photosynthesis is a set of reactions that 'trap' light energy and convert it to the chemical energy of ATP. The second stage of photosynthesis is a set of reactions in which ATP is used to help bond carbon dioxide and water molecules together to create a sugar, such as glucose.

### The first stage of photosynthesis

The first stage of photosynthesis is a set of reactions typically referred to as the light-dependent reactions (see Figure 2.50). In this set of reactions, chlorophyll (and other photosynthetic pigments) absorb light energy and convert that energy to a form of chemical energy, specifically ATP. In addition, light energy is used to accomplish a reaction that is called photolysis of water. In this reaction, a water molecule is split into its component elements: hydrogen and oxygen.



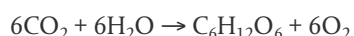
**Figure 2.50** Functions of light during photosynthesis.

The oxygen that is split away due to the photolysis of water is typically released from the plant leaf as a waste product. From the plant's perspective, the useful products formed during this stage of photosynthesis are ATP and hydrogen.

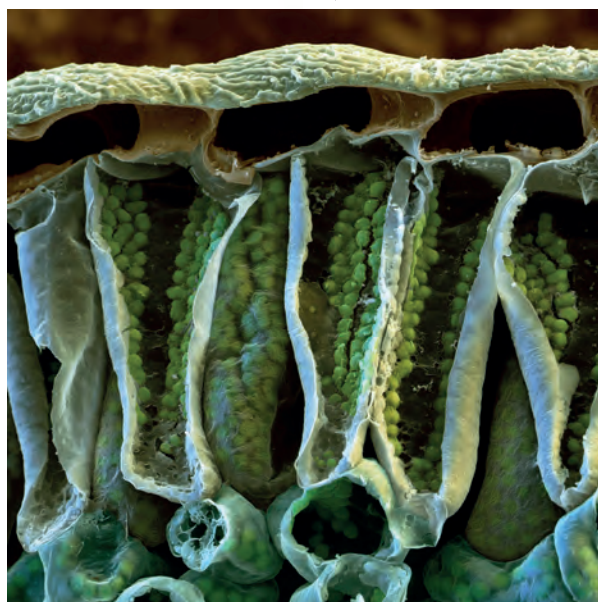
This is an SEM (with false colour added) of an upper leaf section. These cells are very active in photosynthesis, as is shown by the large number of chloroplasts.

### The second stage of photosynthesis

The second stage of photosynthesis is a series of reactions collectively referred to as the light-independent reactions. ATP and hydrogen are used as forms of chemical energy to convert carbon dioxide and water into useful organic molecules for the plant. Glucose, a typical product of photosynthesis, is an organic molecule. It requires six inorganic carbon dioxide molecules to form one glucose molecule.



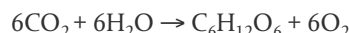
This conversion of an inorganic form of an element to an organic form is known as fixation. Therefore, photosynthesis can be described as a series of reactions in which carbon dioxide and water are fixed into glucose, and oxygen is produced as a by-product.



The fixation reaction described above requires energy. The energy to create the glucose comes directly from the ATP and hydrogen created in the first stage of photosynthesis. Ultimately, this energy can be traced back to sunlight. It is also important to note that glucose is only one of the many possible organic molecules that can be formed from photosynthesis.

## Measuring the rate of photosynthesis

Look again at the summary reaction for photosynthesis:



This balanced equation shows us that carbon dioxide molecules are reactants and oxygen molecules are products of photosynthesis. If you recall some of the information you learned earlier about cell respiration, you will see that the reverse is true for that process. In other words, for cell respiration oxygen is a reactant and carbon dioxide is a product.

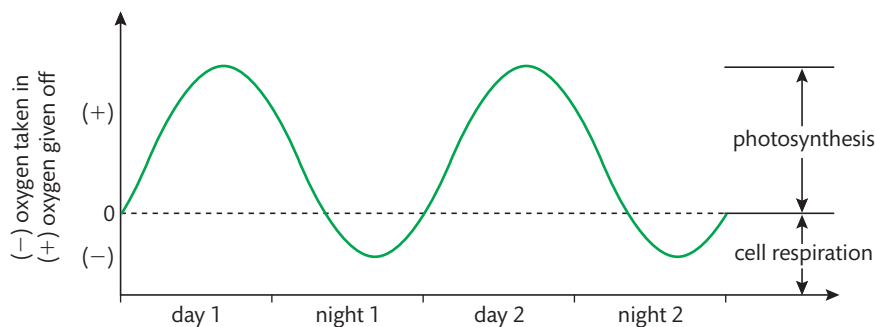
At any given time of year, any one plant has a fairly consistent rate of cell respiration. Not only is this rate consistent throughout the day and night, it is also at a relatively low level. Plants need ATP for various biochemical processes, but the level is typically far lower than any animal needs.

The same consistency is not true regarding the rate of photosynthesis. The photosynthetic rate is highly dependent on many environmental factors, including the intensity of light and air temperature. During the daytime, especially on a warm sunny day, the rate of photosynthesis may be very high for a particular plant. If so, the rate of carbon dioxide taken in by the plant and the rate of oxygen released will also be very high. Because the plant is also carrying out cell respiration, a correction needs to be made for the carbon dioxide and oxygen levels. At night, the rate of photosynthesis may drop to zero. At that time, a particular plant may be giving off carbon dioxide and taking in oxygen to maintain its relatively low and consistent rate of cell respiration (see Figure 2.51).



▲ This student is measuring oxygen produced by an aquatic plant. The rate of oxygen produced is a direct reflection of the rate of photosynthesis.

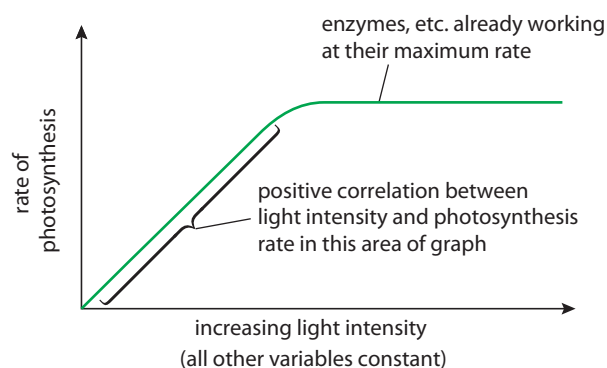
**Figure 2.51** Graph showing the oxygen given off and taken in by a hypothetical plant over a 48-hour period. When the line intersects at 0, the oxygen generated by photosynthesis is equal to the oxygen needed for cell respiration.



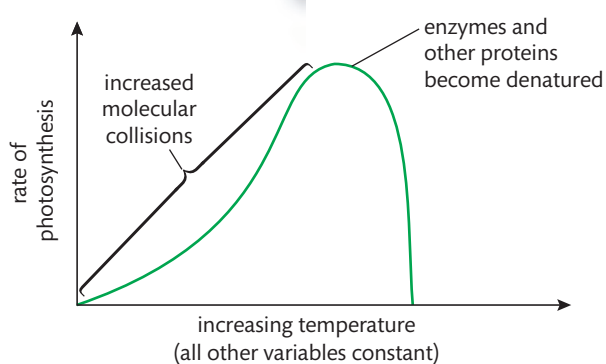
Measuring the rate of oxygen production or carbon dioxide intake is considered to be a direct measurement of photosynthetic rate as long as a correction is made for cell respiration. Another common method for measuring photosynthesis is to keep track of the change in biomass of experimental plants. However, the mass of plants is considered to be an indirect reflection of photosynthetic rate, as an increase or decrease in biomass may be caused by a whole variety of factors as well as the photosynthetic rate.

## The effects of changing environmental factors on the rate of photosynthesis

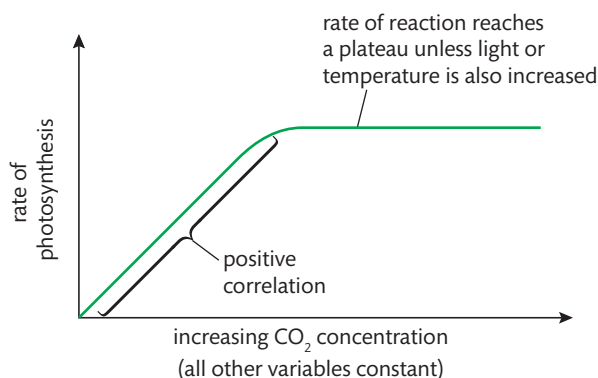
Look now at the patterns that can be seen when three common environmental factors are varied, and how these factors are predicted to change the rate of photosynthesis in a generalized plant (Figures 2.52–2.54).



**Figure 2.52** The effect of increasing light intensity on the rate of photosynthesis.



**Figure 2.53** The effect of increasing temperature on the rate of photosynthesis.



**Figure 2.54** The effect of increasing carbon dioxide concentration on the rate of photosynthesis.

### CHALLENGE YOURSELF

**13** Many scientists have been involved in the development of the concept of limiting factors. They include Justus von Leibig, F. F. Blackmann, and Walter Taylor. A limiting factor is described as a factor that would most directly affect the rate of a physiological process. In photosynthesis, the limiting factor is the one that affects the rate of the photosynthetic process regardless of the effects of other factors. In many cases, it is the one factor that is in 'shortest' supply. Use Figures 2.52–2.54 to answer the following questions about photosynthesis and limiting factors.

- When examining the effect of light intensity on the rate of photosynthesis in Figure 2.52, why is the early part of the graph labelled as a positive correlation?
- In Figure 2.53, why does the denaturing of enzymes and other proteins at high temperatures dramatically lower the rate of photosynthesis?
- In Figure 2.54, what could possibly cause a change from the plateau shown to an increasing rate?
- Design a procedure to investigate the effect of one of the limiting factors mentioned above on the rate of photosynthesis. Some useful information to use in your planning is that water for photosynthesis experiments can be made to be free of dissolved carbon dioxide by boiling and then cooling it.



To learn more about photosynthesis, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.9.



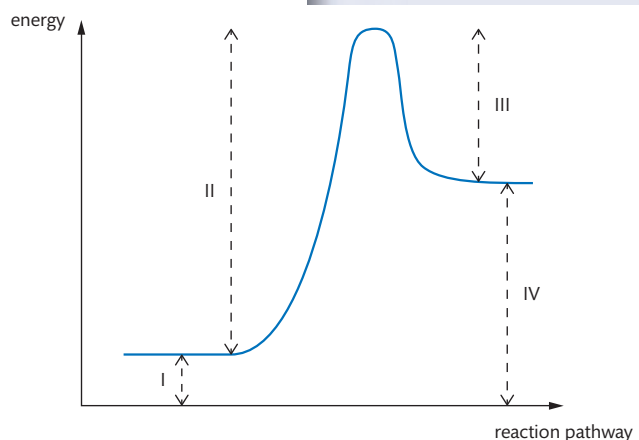
### Exercises

- 25 Explain why a blue object appears to be blue to the human eye.
- 26 Explain why black surfaces (like tarmac and asphalt) get much hotter in sunlight than lighter surfaces (like stone and concrete).
- 27 Plants produce sugars by photosynthesis. What do plants do with the sugars after that?
- 28 Why do most plants produce an excess of sugars in some months of the year?

### Practice questions

- 1 Draw the basic structure of an amino acid, and label the groups that are used in peptide bond formation.

(Total 4 marks)



- 2 The graph to the left shows the energy changes in a chemical reaction.

What would happen to the changes in energy if this reaction was controlled by an enzyme?

- A I would increase.
- B II would decrease.
- C I and IV would decrease.
- D II and III would decrease.

(Total 1 mark)

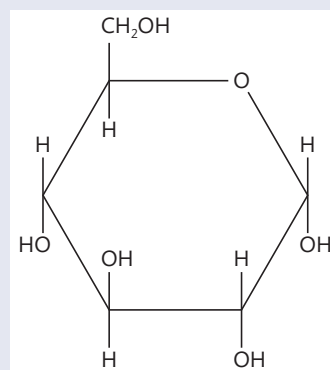
- 3 What causes water to have a relatively high boiling point?
  - A Hydrogen bonds between water molecules.
  - B Hydrogen bonds between hydrogen and oxygen within water molecules.
  - C Cohesion between water molecules and the container in which the water is boiled.
  - D Covalent bonds between hydrogen and oxygen within water molecules.

(Total 1 mark)

- 4 Outline the significance to organisms of the different properties of water.

(Total 5 marks)

5



Which of the following terms correctly describe(s) the molecule above?

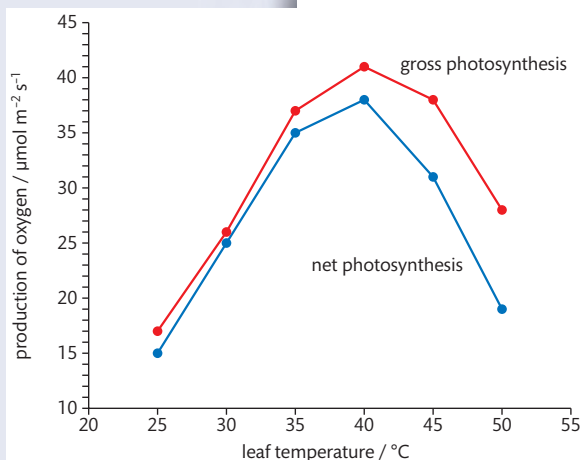
- I Monosaccharide.
  - II Glucose.
  - III Component of triglyceride.
- A I only.
  - B I and II only.
  - C II and III only.
  - D I, II and III.

(Total 1 mark)

- 6 The effect of temperature on photosynthesis was studied in sweet orange, *Citrus sinensis*, using leaf discs. The production of oxygen was used to measure the rate of photosynthesis. Gross photosynthesis refers to the sum of net photosynthesis and respiration. Net photosynthesis was calculated by subtracting the rate of respiration in the dark from gross photosynthesis.

- Identify the optimum temperature for photosynthesis in this plant. (1)
- Determine the difference between gross photosynthesis and net photosynthesis at 40°C and 50°C. (2)
- Deduce what happens to the rate of respiration as the temperature increases between 40°C and 50°C. (1)
- Describe the general pattern of change in photosynthesis in sweet orange as the temperature increases. (1)
  - Compare the effect of temperature on photosynthesis with the effect of temperature on respiration in sweet orange. (2)

(Total 7 marks)

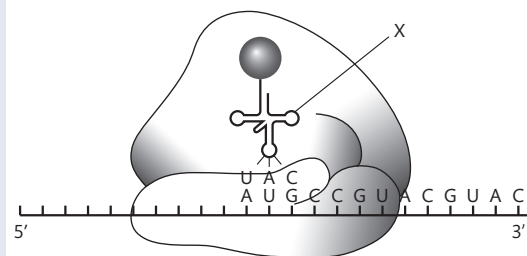


Ribeiro et al. 2006

- 7 What sequence of processes is carried out by the structure labelled X during translation?

- Combining with an amino acid and then binding to an anticodon.
- Binding to an anticodon and then combining with an amino acid.
- Binding to a codon and then combining with an amino acid.
- Combining with an amino acid and then binding to a codon.

(Total 1 mark)



- 8 The diagram to the right shows part of the respiratory pathway. The number of carbon atoms in each molecule is indicated.

- Label pyruvate and acetyl coenzyme A on the diagram. (1)
  - Indicate **two** places where decarboxylation occurs on the diagram. (1)
  - List **one** product other than carbon dioxide formed in this stage of respiration. (1)
- State precisely where in a cell this stage of respiration is occurring. (1)

(Total 4 marks)

